Serum Integrin β 1 Levels as a Prognostic Marker in Metastatic Colorectal Cancer

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Abstract

Aims : In this study, we examined whether the integrin β 1 subunit, thought to play an important role in colorectal cancer invasion and metastasis, can be used as a prognostic marker for colorectal cancer.

Patients and Methods : The subjects of this study were 86 patients who underwent surgery for colorectal cancer at this department. We measured preoperative serum integrin β 1 levels and conducted a 5 year retrospective postoperative study, examining the relationship between integrin β 1 levels and survival curves.

Results: For patients with postoperative recurrence or metastasis, outcomes were significantly better in those with a preoperative serum integrin β 1 level $\geq$ 600 ng/mL than those with $<$ 600 ng/mL ($p = 0.0062$).

Conclusions: Even in patients with metastases identified preoperatively, outcomes were favourable if preoperative serum integrin β 1 levels were high. These results indicate that integrin β 1 shows promise as a prognostic marker in metastatic colorectal cancer.

Key words: integrin β 1, prognostic marker, recurrence and metastasis of colorectal cancer

Introduction

Integrins are cell adhesion molecules that play a role in cancer invasion and metastasis, as well as inflammatory processes¹⁻⁴⁾. Researchers are now investigating clinical applications of integrin, such as integrin α IIb β 3 inhibitors in the treatment of coronary arterial disease⁵⁻⁷⁾.

Integrin subunit β 1 has been reported to play a major role in colorectal cancer invasion and metastasis^{1,2,8-10)}. Hayashi of this Department, reported that negative staining for α 6 β 1 integrin may be a risk factor for colorectal cancer metastasising to the liver¹¹⁾. Soyama et al. (also of this Department) reported lower serum levels of integrin β 1 and negative immunohistological staining in patients with advanced colorectal cancer¹²⁾.

In this retrospective study, we conducted a follow-up survey of patients following surgery for colorectal cancer, comparing preoperative serum integrin β 1 levels in cases of recurrence, metastasis and death. We then examined whether preoperative integrin β 1 levels can be used clinically as a prognostic marker for colorectal cancer.

Patients and Methods

1 Patients

The subjects of this study were 86 patients who underwent surgery for colorectal cancer at the Department of Surgery II, Tokyo Women's Medical University, between April 1996 and May 1997.

Subjects comprised 56 males (average age 62.77 years) and 30 females (60.33 years). The site of the primary lesion was the colon in 45 patients and the rectum in 41. The histological depth of invasion was

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Table 1 Serum integrin $\beta 1$ levels and outcomes according to stage

stage	suivivors without recurrence	suivivors with recurrence	cancer deaths
0	—	—	—
I	528.5 $\pm$ 240.0	—	—
II	371.5 $\pm$ 302.7	979.5 $\pm$ 297.7	485
IIIa	349.8 $\pm$ 205.4	461.7 $\pm$ 190.7	387.8 $\pm$ 176.9
IIIb	501.5 $\pm$ 277.5	—	355.0 $\pm$ 153.1
IV	—	927.5 $\pm$ 98.3	343.7 $\pm$ 295.0

(ng/mL)

m in 6 patients, sm in 4, mp in 11, ss(a1) in 41, and se(a2) in 24. The clinical staging was 0 in 6 patients, I in 10, II in 17, IIIa in 26, IIIb in 10, and IV in 17.

2 Study Methods

(1) Measurement of serum integrin $\beta 1$ levels

Venous blood samples were collected from patients prior to surgery, the serum separated using a centrifuge and preserved at -20°C . Samples were defrosted prior to measurements.

Measurements were made using a 2 step sandwich EIA method with a Fibronectin receptor (FNR) kit (Takara, Shiga, Japan). First, 200 μL /well antibody was added to 96-well EIA plates, coating the antibody overnight at 4°C . Blocking was then performed by adding 200 μL /well blocking liquid to each plate, and incubating for 2 hours at 37°C . Further 100 μL /well antibodies were then added, and reacted for one hour at room temperature, comprising the first reaction. This was followed by the addition of 100 μL /well antibody marker, which was reacted for one hour at room temperature, comprising the second reaction. Then 100 μL /well substrate liquid was added, and reacted for 15 minutes at room temperature, comprising the third reaction. Finally, NH_2SO_4 100 μL /well was added to stop the reaction. Absorbance was measured at a wavelength of 492 nm using a microplate reader, and the concentration of each antibody was read from the standard curve.

(2) Follow-up of outcomes

All 86 patients were followed for 5 years after their initial surgery. They were surveyed for disease-free survival, recurrence and metastasis survival, cancer death, death from other causes, and postoperative treatment.

(3) Outcomes and serum integrin $\beta 1$ levels

1. Based on the results of (2) above, we examined the relationship between outcomes and preoperative integrin $\beta 1$ levels.

2. We also examined the relationship between 5 year survival rates and high and low preoperative integrin $\beta 1$ levels.

Clinical pathological findings were recorded in accordance with the 'General rules for clinical and pathological studies on cancer of the colon, rectum and anus The 5th edition'¹³⁾.

Statistical analyses were performed with Stat View 5.0 software, using non-parametric analysis (Mann-Whitney U test), and the logrank test for Kaplan-Meier survival rates, with $p < 0.05$ considered to denote a statistically significant difference.

Results

(1) Preoperative serum integrin $\beta 1$ levels and clinicopathological findings

Serum integrin $\beta 1$ levels were previously reported by Soyama et al. The mean preoperative serum integrin $\beta 1$ level was 495.8 ± 391.6 ng/mL in our 86 patients with colorectal cancer, and 450.9 ± 349.5 ng/mL in 39 healthy controls. No significant difference was seen between groups. As the cancer invasiveness increased, the serum integrin $\beta 1$ level decreased. Serum integrin $\beta 1$ levels decreased significantly as the depth of invasion increased, as metastasis to lymph nodes progressed, as lymphatic invasion progressed, and as the histological stage advanced, but no significant change was seen with venous invasion.

(2) Follow-up of outcomes

Of the 86 patients, 12 patients were lost to follow-up or died from other causes. Follow-up for 5 years after initial surgery for colorectal cancer yielded 50 survivors, of whom 43 were free of recurrence, and 7 had experienced recurrence. Disease free survival was enjoyed by 4 patients with stage 0 disease initially, and 8 with stage I disease, and there were no cancer deaths. Of the patients with stage II disease initially, 10 were free of recurrence, 2 had experi-

Fig. 1 Comparison of survival rates between subjects with high and low serum integrin $\beta 1$ levels

Fig. 2 Comparison of 5 year survival rates between high and low serum integrin $\beta 1$ levels in subjects with recurrence and metastasis

enced recurrence, with 1 cancer death. Of the patients with stage IIIa disease initially, 17 were free of recurrence, 3 had experienced recurrence, with 4 cancer deaths. Of the patients with stage IIIb disease initially, 4 were free of recurrence, and no surviving patients had experienced recurrence, with 5 cancer deaths. No patients with stage IV disease initially remained free of recurrence, 2 had experienced recurrence, with 14 cancer deaths.

(3) Serum integrin $\beta 1$ levels and outcomes

1. **Table 1** shows the relationship between serum integrin $\beta 1$ levels and outcomes according to stage. The mean serum integrin $\beta 1$ level in survivors with stage IV disease was high at 947.5 ± 98.3 ng/mL.

2. Serum integrin $\beta 1$ levels and 5 year survival rates

Comparison of 5 year survival rates between high and low serum integrin $\beta 1$ levels in all patients revealed a tendency to poorer outcomes in patients with levels <600 ng/mL than for ≥ 600 ng/mL, although the difference was not significant ($p = 0.0845$) (**Fig. 1**).

Fig. 2 shows a comparison of 5 year survival rates between high and low serum integrin $\beta 1$ levels in survivors with recurrence and metastasis, and cancer deaths. A significant difference was seen in outcomes between patients with serum integrin $\beta 1$ levels <600 ng/mL and those with ≥ 600 ng/mL ($p = 0.0062$).

Discussion

Surgical procedures for primary colorectal cancers are now fairly well established, and yield favourable results. The most important factors affecting outcomes are therefore postoperative metastasis and recurrence, and how metastatic lesions are treated^(7,9).

In recent years, the relationship between local invasion and metastasis by cancers and cell adhesion molecules has attracted considerable attention. The process of local invasion and metastasis begins when cancer cells leave the original tumour and enter a blood or lymphatic vessel, then adhere to vascular endothelial cells within a distant organ, leave the vessel, and begin to proliferate within the distant organ^(14,15). Cell adhesion molecules may control these processes, so we examined whether these molecules can act as prognostic factors for cancer, or have applications in treatments to inhibit cancer recurrence and metastasis.

Integrins, cell adhesion molecules present on the cell surface, are receptors that interact with the extracellular matrix (ECM). They are heterodimers containing two chains, the α and β subunits. At present, 19 α subunits and 8 β subunits have been discovered, and at least 24 combinations identified^(1,2,16).

Of all the integrins present in the human body, the $\beta 1$ subunit is thought to be the most abundant. At present, 12 integrins containing the $\beta 1$ subunit have been identified. This $\beta 1$ subfamily is thought to play an important role in cancer invasion and metastasis because they are strongly involved in attachment of cancer cells to the basement membrane, acting as receptors to ECM structural components such as laminin, fibronectin and type IV collagen^(3,8).

At this Department, our studies have concentrated on adhesive molecules in colorectal cancer. Saito et al. studied laminin, finding that positive serum laminin and negative laminin staining are risk factors and useful predictive factors for colorectal cancer metastasis to the liver^(17,18). From the $\beta 1$ subfamily, Hayashi focused on integrin $\alpha 6 \beta 1$ (VLA-6), a laminin specific receptor. The results of immunohistological staining indicated that negative staining for integrin $\alpha 6 \beta 1$ is a risk factor for hepatic metastasis.

Soyama has focused on the $\beta 1$ subunit, measuring serum levels and performing immunohistological staining. He has found low serum integrin $\beta 1$ lev-

els and negative immune staining in patients with advanced colorectal cancer. These results suggest that these 2 tests may be useful in assessing the degree of progression of colorectal cancer⁽¹²⁾.

Integrins containing a $\beta 1$ subunit ($\alpha 1 \beta 1$, $\alpha 2 \beta 1$, $\alpha 3 \beta 1$ and $\alpha 5 \beta 1$) and integrin $\alpha 6 \beta 4$ are expressed in the normal colorectal mucosa. Various changes are seen in these integrins in patients with colorectal cancer. Koretz et al. reported decreased expression of integrin $\alpha 2 \beta 1$ in patients with colorectal cancer, and Pignatelli et al. reported decreased levels of integrins $\alpha 2 \beta 1$ and $\alpha 3 \beta 1$ in patients with moderately differentiated colorectal adenocarcinoma^(19,20). Stallmach et al. also reported decreased expression of integrins $\alpha 2 \beta 1$, $\alpha 3 \beta 1$ and $\alpha 5 \beta 1$ in colorectal cancer in comparison to healthy mucosa, and the decrease was greater in poorly differentiated and advanced disease⁽⁴⁾.

Our results show that with progression of colorectal cancer, integrin $\beta 1$ serum levels and immunohistological staining in the primary lesion decrease. This suggests that changes in integrin expression associated with cancer cell proliferation and metastasis change the adhesiveness between cancer cells, and between cancer cells and ECM proteins, giving rise to a state where cancer cells readily detach from the primary lesion.

Different cancer cell lines express different integrins to different degrees. For instance, integrin $\alpha 3 \beta 1$ is involved in cell migration rather than adhesion. Expression of integrin $\alpha 3 \beta 1$ in malignancies is heterogenous, and has been reported to be reduced in colorectal and pancreatic cancers, but unchanged in lung cancers⁽¹⁸⁾.

Now we will turn our attention to the fact that the mean serum integrin $\beta 1$ level in 5 year survivors with stage IV disease was high at 947.5 ± 98.3 ng/mL.

All of the longterm survivors of this study underwent chemotherapy or surgical treatment for recurrent or metastatic disease if metastases were present at the time of initial surgery. This suggests that if serum integrin $\beta 1$ levels prior to initial surgery are high, even if metastases are already present at that time, the prognosis may be favourable due to inhibition of further metastases, or enhanced sensitivity to chemotherapy.

An interesting feature of this study is the comparison between patients who survived 5 years

without recurrence and those who died of their cancer during the follow-up period. Although subject numbers were low, significantly more patients survived with an initial serum integrin $\beta 1$ level above the cutoff value of 600 ng/mL. We conjectured that patients with high serum integrin $\beta 1$ levels at the time of surgery can expect favourable outcomes, even if metastatic disease was already present at that time.

Giancotti *et al.* and Schreiner *et al.* reported that overexpression of integrin $\alpha 5 \beta 1$ was induced when transformed Chinese hamster ovary (CHO) cells were transfected with integrin $\alpha 5 \beta 1$ genes, decreasing the CHO cells' mobility and their tumorigenicity in nude mice. Furthermore, the growth rates of CHO cells with different levels of integrin $\alpha 5 \beta 1$ expression, placed subcutaneously in nude mice, correlated inversely with the expression of $\beta 1$ integrin^{10,21)}.

Fujita reported that antibodies to the integrin $\beta 1$ subunit strongly inhibit cancer cell adhesion to and invasion of hepatic tissue, hepatic metastasis and peritoneal dissemination in a model of cancer cell adhesion to hepatic tissue, an in vitro invasion model, an in vivo hepatic metastasis model, and a peritoneal dissemination model, although inhibition was not seen with other subunits, $\beta 4$ and $\alpha 1$ ^{22,23)}.

In this Department, we conducted a study of the use of cell adhesion domains of adhesion molecules as inhibitors of metastasis. Using a mouse hepatic metastasis model, we achieved approximately 50% inhibition of metastasis with a competitive inhibitor of integrin on the cancer cell surface²⁴⁾.

The results of this study raise the prospect of clinical applications, as raising serum integrin levels may further inhibit metastasis and recurrence, and increase chemotherapy sensitivity. Serum integrin levels and the degree of immune staining at the time of detection of the primary tumour may also be useful in predicting therapeutic response and outcomes^{9,22)}.

Conclusions

We conducted a 5 year follow-up survey of 86 patients in whom we had measured preoperative serum integrin $\beta 1$ levels prior to surgery for colorectal cancer, reaching the following conclusions.

1. For all disease stages, 5 year survival curves showed that outcomes tended to be poorer in pa-

tients with lower preoperative serum integrin $\beta 1$ levels.

2. In patients with postoperative recurrent and metastatic disease, 5 year survival rates were significantly higher in patients with higher preoperative serum integrin $\beta 1$ levels.

3. Our results indicate that preoperative integrin $\beta 1$ levels may be a useful prognostic marker for colorectal cancer.

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