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The Significance of the Ki-67 Labeling Index, the Expression of c-kit, p53, and bcl-2, and the Apoptotic Count on the Prognosis of Gastrointestinal Stromal Tumor

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1. Introduction

Gastrointestinal stromal tumor (GIST) express the cell surface transmembrane receptor KIT that has tyrosine kinase activity and is the protein product of the KIT proto-oncogene *c-kit*. Activation or gain-of-function mutation in the *c-kit* gene has been identified in the majority of GIST cases.

The mutation results in the constitutive activation of KIT signaling, which leads to uncontrolled cell proliferation and resistance to apoptosis. Somatic mutation in the *c-kit* gene of GIST has been reported to be associated with aggressive progression and poor prognosis. The diameter of the tumor and the mitotic index have been reported as useful indices of the biological malignancy of GISTs. However, a few cases in the benign group had high cellularity, ulcer formation or metastasis/recurrence. Therefore, other indices of the biological malignancy of GISTs are thought to be needed.

Recently, the Ki-67 labeling index, p53 immunoreaction, and bcl-2 overexpression have each been reported to be useful in predicting the potential malignancy in GISTs. In this chapter, the reliabilities of the Ki-67 labeling index, the expression of c-kit, p53, and bcl-2, and the apoptotic count for predicting potential malignancy were assessed.

2. Patients and methods

2.1 Patients

Twelve patients with gastric or small intestine stromal tumors who underwent surgical resection at our department; 11 involving the stomach and the other one involving the small intestine, were retrospective analyzed. Nine patients were male, and three were female (age range, 47-79 years; mean age, 62.8±10.5 years). The series consisted of seven patients with GIST in the upper body of the stomach, one in the upper body of the remnant stomach, two in the middle body of the stomach, one in the antrum, and one in the jejunum. Four patients who either had metastasis at the time of the initial operation or recurrence after operation were classified as the metastasis/recurrence group, whereas the other eight patients are surviving without postoperative recurrence.

2.2 Clinicopathological study

As prognostic determinants, we examined mean tumor diameter, and the mitotic index (the frequency of mitosis in 50 visual fields at a magnification of $\times 400$). We used Amin's classification of malignant (mitotic index $>5/50$ high power fields (HPF), irrespective of tumor diameter), borderline (mitotic index $<5/50$ HPF and tumor diameter >5 cm), and benign (mitotic index $<5/50$ HPF and tumor diameter <5 cm).

2.3 Immunohistochemical staining

After an initial review of all available hematoxylin and eosin (H&E)-stained slides of the surgical specimen, we selected paraffin blocks in which the central region of the tumor was clearly revealed, from each case, to study. Serial 4- μ m-thick sections were recut from each block. One section from each block was stained with H&E. Other sections were immunostained for c-kit, CD34, vimentin, α -smooth muscle stain (SMA), desmin, S-100, Ki-67, p53, and bcl-2. Immunostaining was performed using the dextran polymer method for c-kit, CD34, α -SMA, desmin, and S-100. Anti-human c-kit rabbit polyclonal antibody (DAKO, Kyoto, Japan), anti-human CD34 mouse monoclonal antibody (DAKO), anti-human vimentin mouse monoclonal antibody (DAKO), anti-human α -SMA mouse monoclonal antibody (DAKO), anti-human desmin mouse monoclonal antibody (DAKO), or anti-human S-100 rabbit polyclonal antibody (DAKO) was used as the primary antibody. The deparaffinized sections were irradiated by microwaves for 15 min (400W; H2800 microwave processor, Energy Beam Sciences) at 90°C and were incubated with 0.03% H₂O₂ / methanol for 30 minutes for blocking endogenous peroxidase activity. After washing in phosphate buffered saline (PBS), the sections were incubated with the primary antibody for 30 minutes at room temperature. After rewashing, the sections were further incubated with peroxidase-labeled dextran conjugated anti-mouse and anti-rabbit immunoglobulin goat polyclonal antibody (DAKO) for 30 min at room temperature. After washing again, the sections were developed with chromogen 3,3'-diaminobenzidine tetrahydrochloride.

Immunostaining was performed using the avidin-biotin complex method for Ki-67, p53, and bcl-2. Anti-human Ki-67 mouse monoclonal antibody (MM1 Novocastra, Newcastle, UK), anti-human p53 mouse monoclonal antibody (Do-7 Novocastra), or anti-human bcl-2 mouse monoclonal antibody (Novocastra) was used as the primary antibody. The deparaffinized sections were heated in an autoclave at 120°C for 5 minutes in citric acid buffer (2 mmol/l citric acid and 9 mmol/l trisodium citrate dehydrate, pH6.0) and incubated with 0.03% H₂O₂ / methanol for 30 minutes for blocking endogenous peroxidase activity. After washing in PBS, nonspecific binding was blocked by incubating the sections with normal animal serum for 20 minutes. The sections were incubated with the primary antibody overnight at 4°C. After washing, the sections were further incubated with biotinylated second antibody for 30 minutes at room temperature. The primary antibody was detected using the avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA) and 3-amino-9-ethylcarbazole as the chromogen. These thin sections were also counterstained with hematoxylin and mounted. For immunoreactivity of p53 and bcl-2 proteins, tissue sections with $>10\%$ immunopositive cells in 1000 tumor cells from three arbitrary microscopic fields were defined as being positive (+), and those with $<10\%$ immunoreactive tumor cells were defined as being negative (-).

The Ki-67 Index was defined as the percentage of tumor cells displaying immunoreactivity in 1000 cells in a $\times 100$ magnified field from five arbitrary microscopic fields.

2.4 TUNEL staining

Apoptotic tumor cells were detected by using the terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate biotin nick-end labeling (TUNEL) method. The Apo Tag *in situ* detection kit (Intergen, Purchase, NY) was used according to the manufacturer's instructions. The apoptotic count was defined as the mean number of TUNEL-positive cells in a $\times 400$ magnified field from 10 arbitrary microscopic fields.

2.5 Statistical analysis

Student's *t* -test and the chi-square test were used to analyze the data for significant differences, and differences were considered statistically significant when $P < 0.05$. Continuous variables were presented as the mean \pm SD.

3. Clinicopathological study

3.1 Clinicopathological features

In six cases, the lesion was incidentally discovered during a routine screening examination. The other six cases were symptomatic and presented epigastric pain, abdominal discomfort, anemia, vomiting, an abnormal shadow on chest X-ray, or tumor palpable, respectively. The lesion size ranged from 18 to 200 mm in diameter (mean size 55.0 ± 44.5 mm). The GIST in the jejunum was palpable. The macroscopic aspects of GIST in the jejunum are a smooth surface, gray color, pseudocapsule, and exophytic growth. We found one case with liver metastasis, one with lung metastasis, one with bone metastasis, and no cases with lymph node metastasis at the time of surgery (Table 1).

3.2 Histology and malignancy

A Mitotic Index of $> 5/50$ HPF was seen in five cases and $< 5/50$ HPF in the other seven cases. A high cellularity was seen in four cases, a middle cellularity in four cases, and a low cellularity was seen in the other four cases. Concerning the degree of anaplasia, a high degree was seen in three cases, a middle degree in four cases, and a low degree was seen in the other five cases. The number of cases with ulcer formation, cases with necrosis, cases with bleeding in the tumor, and the number of cases with mucosal invasion by tumor cells was four, one, four, and one, respectively. The numbers in the malignant group, borderline group, and in the benign group were five, two, and five, respectively. Three of four cases in the metastasis/recurrence group were malignant group, but other one was benign group. All four cases in the metastasis/recurrence group had ulcer formation and high cellularity. One case with necrosis and mucosal invasion had lung metastasis. Two of four cases with bleeding in the tumor was in the metastasis/recurrence group. Two of three cases with high atypia was in the metastasis/recurrence group. In the malignant group, the tumor size was > 5 cm in diameter in three cases, the cellularity in four cases was middle or high, three cases had ulcer formation, three cases had bleeding in the tumor, and one case had necrosis in the tumor with mucosal invasion by tumor cells. In the borderline group, the cellularity was middle or low, the degree of anaplasia was high or low, one case had bleeding in the tumor, and there was no ulcer formation, no necrosis in the tumor, and no mucosal invasion. In the benign group, the tumor size was 3 cm or less in diameter. The cellularity was low in two cases, middle in two cases, and high in one case. The degree of anaplasia was low in three cases, middle in two cases, and high in no case. One case with bone metastasis had ulcer

formation . There was no necrosis, no bleeding in the tumor, and no mucosal invasion (Table 1).

| Case | Site | Mitosis(HPF) | Size(mm) | Cellularity | Ulcer | Necrosis | Bleeding | Atypia | Invasion | *Metastasis | Malignancy |
|------|------|--------------|-------------|-------------|-------|----------|----------|--------|----------|-------------|------------|
| 1 | U | 10/10 | 80x60 | High | + | - | - | High | - | Liver | Malignant |
| 2 | U | 6/50 | 40x33 | Middle | - | - | + | Low | - | None | Malignant |
| 3 | M | 6/50 | 18x12 | Low | - | - | - | Middle | - | None | Malignant |
| 4 | U** | 13/10 | 120x100 | High | + | + | + | High | + | Left lung | Malignant |
| 5 | U | - | 52x48 | Middle | - | - | + | High | - | None | Borderline |
| 6 | U | 4/50 | 60x45 | Low | - | - | - | Low | - | None | Borderline |
| 7 | U | - | 30x30 | Low | - | - | - | Low | - | None | Benign |
| 8 | L | - | 30x30 | Middle | - | - | - | Middle | - | None | Benign |
| 9 | U | 3/50 | 25x25 | High | + | - | - | Middle | - | bone | Benign |
| 10 | M | - | 25x20 | Low | - | - | - | Low | - | None | Benign |
| 11 | U | 3/50 | 20x20 | Middle | - | - | - | Low | - | None | Benign |
| 12 | J | 10/10 | 200x160x120 | High | + | - | + | Middle | - | None | Malignant |

U: upper body of the stomach, M: middle body of the stomach, L: lower body of the stomach, J: jejunum, *mucosal invasion, **upper part of the remnant stomach

Table 1. Histological findings and malignancy

The diameter of the tumor and the mitotic index have been reported as useful indices of biological malignancy in GISTs. We used Amin’s classification, preparing evaluation criteria based on the pattern of mitosis and the diameter of the tumor. Mucosal invasion, bleeding, necrosis, and high degree of anaplasia were not recognized in the benign group. However, one case with bone metastasis in benign group had high cellularity and ulcer formation. Therefore, the diameter of the tumor and the mitotic index were not only useful indices of biological malignancy, but also cellularity, ulcer formation, necrosis, bleeding in the tumor, anaplasia, and mucosal invasion.

3.3 Treatment

Concerning the treatment, local resection was performed in eight cases, and in five of these cases, laparoscopic local resection was performed. In the other four cases, proximal gastrectomy with intrahepatic arterial infusion of adriamycin, resection of the remnant stomach with left lower lobectomy of the lung, local resection with radiation for bone metastasis and partial resection of the jejunum including the tumor were performed (Table 2).

3.4 Prognosis

Concerning survival, one patient with lung metastasis received resection of the remnant stomach with left lower lobectomy of the lung, and later died of intra-abdominal and right lung recurrence at 35 months later. One patient with multiple liver metastasis received proximal gastrectomy with intrahepatic arterial infusion of adriamycin, recurrence in the abdomen was recognized, removed and imatinib was administered from 41 months, but

died of peritoneal metastasis at 58 months after the first surgery. The prognosis of the patient with bone metastasis received local resection with radiation was unknown. The patient with the large GIST in the jejunum received partial resection of the jejunum including the tumor, recurrence in the liver was recognized and imatinib was administered from 25 months, local recurrence was recognized in the small intestine and removed at 45 months, and was still alive at 75 months after the first surgery (Table 2). The other patients are still alive and disease-free.

| Case | Age | Sex | Treatment | Recurrence | Imatinib | Prognosis |
|------|-----|-----|--|------------------------------------|----------|------------|
| 1 | 49 | M | Proximal gastrectomy Intrahepatic arterial infusion* | 41M peritoneum | + | 58M dead |
| 2 | 68 | M | Laparoscopic local resection | - | - | 8M alive |
| 3 | 63 | M | Laparoscopic local resection | - | - | 120M alive |
| 4 | 55 | M | Resection of the remnant stomach Lt. lower lobectomy of the lung | 30M peritoneum,lung | - | 35M dead |
| 5 | 64 | M | Laparoscopic local resection | - | - | 79M alive |
| 6 | 66 | F | Local resection | - | - | 8M alive |
| 7 | 70 | F | Laparoscopic local resection | - | - | 8M alive |
| 8 | 47 | M | Local resection | - | - | 128M alive |
| 9 | 78 | F | Local resection, Radiation | - | - | - - |
| 10 | 51 | M | Laparoscopic local resection | - | - | 49M alive |
| 11 | 63 | M | Local resection | - | - | 34M alive |
| 12 | 79 | M | Partial resection of jejunum Tumor resection | 25M Liver, 45M Local recurrence | + | 75M alive |

*: Intrahepatic arterial infusion of adriamycin

Table 2. Treatment and prognosis

4. Immunohistochemical staining

4.1 c-kit expression

All patients were positive for vimentin expression. Seven patients were positive for c-kit expression. Nine patients were positive for CD34 expression. In the seven c-kit-positive cases, six patients were also positive for CD34 expression. Two patients were positive for α-SMA expression. All patients were negative for both desmin and S-100 expression. All four cases in the metastasis/recurrence group were positive for c-kit expression (Table 3). The positive rate of c-kit in the malignant group was 100% (five of five), in the borderline group, it was 50% (one of two), and in the benign group, it was 20% (one of five; Table 4)).The positive rate of c-kit in the malignant group was significantly higher than in the borderline and benign group ($P < 0.05$). Mean tumor size in the c-kit -positive group was 76.4 ± 62.2 mm and c-kit-negative group was 33.0 ± 21.0 mm (Table 5).The mean tumor diameter in the c-kit-positive group was higher than that in the c-kit-negative group. Concerning any correlation between cellularity and c-kit expression, the positive rate of c-kit in the high group was 100% (four of four), in the middle group was 50% (two of four), and in the low group was 25% (one of four). Concerning any correlation between the degree of anaplasia and the c-kit expression, the positive rate of c-kit in the high group was 100%

(three of three), in the middle group was 75% (three of four), and in the low group was 20% (one of five). The positive rate of c-kit in cases with ulcer formation was 100% (four of four), and in cases without ulcer formation it was 37.5% (three of eight). The positive rate of c-kit in cases with bleeding in the tumor was 100% (four of four), and in cases without bleeding in the tumor, it was 37.5% (three of eight). All malignant cases, cases with high cellularity, cases with high degree of anaplasia, cases with ulcer formation, and cases with bleeding in the tumor were positive for c-kit expression.

Immunohistochemistry analyses have been reported to show a positivity reaction to CD34 in > 80% of cases, and to the c-kit in 95-100%. In our study, the positive rate to CD34 was 83.3%, and to the c-kit it was 66.7%. Therefore, the positive rate to the c-kit was lower than in other reports. However, all other reported cases lacked immunohistochemical evidence of smooth muscle or neural differentiation. Hirota reported that some GISTs lacked immunoreactivity to c-kit without immunohistochemical evidence of smooth muscle or neural differentiation. Many GISTs showed a positivity to CD34 recognized in endothelial cells and in interstitial cells of Cajal. The positive rate to α -SMA was 16.7% in our cases. Hirota reported that from 20 to 30% of GISTs were positive to α -SMA. Somatic mutation in the c-kit gene of GIST has been reported to be associated with aggressive progression and poor prognosis. Ernst reported that the 3-year survival rate of cases without mutation in c-kit was >65%, but that of cases with mutation in c-kit it was < 30%. In our cases, all malignant cases were c-kit positive, and in the benign group only one case which had bone metastasis was c-kit positive. The average diameter in the c-kit-positive group was higher than that in the c-kit-negative group.

The expression of c-kit was considered to reflect the malignancy of the GIST. It has recently been clarified that the autoactivation of tyrosin kinase caused by upregulation of the c-kit gene was related essentially to proliferation in the GIST cells, and imatinib, which inhibits the autoactivation of tyrosine kinase, has been reported to be effective as a molecule-targeting treatment. Although adjuvant use of imatinib is currently under evaluation, it is expected to be a useful treatment for an unresectable GIST or for a patient with metastasis/recurrence.

4.2 The Ki-67 labelling index

Immunoreactivity to Ki-67 was seen in the nucleus of tumor cells (Fig. 1). The Ki-67 labeling index ranged between 4.7 to 49.8 (mean 25.4 ± 15.9 ; Table 3). In the metastasis/recurrence group, the Ki-67 labeling index in patient with liver metastasis was 49.8, in patient with bone metastasis was 26.8, in patient with lung metastasis, it was 40.2, and in small intestinal GIST patient with recurrence in the liver, it was 34.6. The Ki-67 labeling index in the malignant group was 35.3 ± 11.0 , in the borderline group 37.5 ± 9.40 , and in the benign group was 10.6 ± 9.26 (Table 4). The Ki-67 labeling index in the malignant group was significantly higher than that in the benign group ($p < 0.01$). The Ki-67 labeling index in the c-kit-positive cases was 35.3 ± 10.2 and in c-kit-negative cases was 11.4 ± 11.0 . The Ki-67 labeling index in the c-kit positive cases was significantly higher than that in the c-kit-negative cases ($p < 0.01$).

The Ki-67 labeling index in the c-kit-positive cases was 35.3 ± 10.2 and in c-kit-negative cases was 11.4 ± 11.0 (Table 5). The Ki-67 labeling index in the c-kit positive cases was significantly higher than that in the c-kit-negative cases ($p < 0.01$).

AgNOR staining, DNA ploidy pattern of the nucleus, bromodeoxyuridine labeling index, Ki-67 labeling index, proliferative cell nuclear antigen (PCNA), and p53 staining have each

been reported to be a useful marker as an index of malignancy in GIST. Especially, the Ki-67 labeling index has recently been used as an excellent index of cell growth. The mitotic index reflects the M stage of mitosis only; however, because the Ki-67 labeling index can recognize most proliferating cells in stages G1, S, and G2, it is considered to be more appropriate as an objective index of the malignancy in GIST. Shimoda et al. reported that the Ki-67 labeling index was $> 10\%$ in all patients with a mitotic index $> 10/200$ HPF. Wang et al. reported that the prognosis of GIST was significantly poor when the Ki-67 labeling index was 10% or more. Nagasako et al. reported that the maximum tumor diameter, mitotic index, and Ki-67 labeling index were useful as indices of malignancy for a gastric stromal tumor. In the present study, all four cases of the metastasis/recurrence group showed a high Ki-67 labeling index (49.8, 26.8, 40.2 and 34.6), and the mean Ki-67 labeling index in the malignant group was higher than that in the benign group. Even in the benign group, one case with bone metastasis showed a high Ki-67 labeling index (26.8), and this case showed high cellularity and had ulcer formation. Such a case should be carefully followed postoperatively. The mean Ki-67 labeling index in the c-kit-positive cases was higher than that in the c-kit-negative cases. The Ki-67 labeling index was considered to be a useful marker of malignancy in GIST.

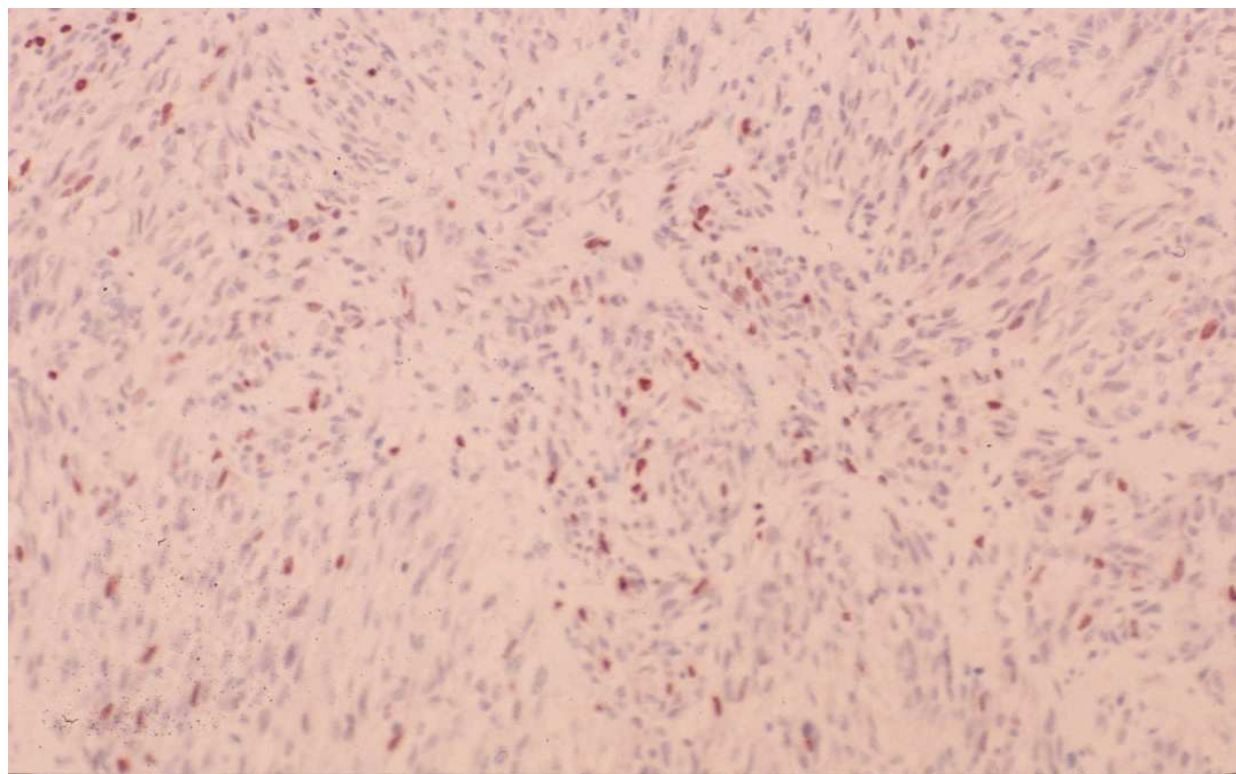


Fig. 1. Immunoreactivity for Ki-67; Immunostaining for Ki-67 was confined to almost the entire nucleus ($\times 200$)

4.3 p53 expression

The immunoreactivity of p53 was also mainly identified in the nucleus (Fig. 2). Four patients were positive for p53 expression. Three cases of the metastasis/recurrence group were positive for p53 expression. The positive rate of p53 in the malignant group was 60.0% (three

of five), in the borderline group was 50.0% (one of two), and in the benign group was 0% (zero of five; Table 4). The mean tumor size in the p53-positive group was 115.0 ± 61.9 mm and in the p53-negative group was 26.3 ± 11.9 mm. The mean tumor size in the p53-positive group was higher than that of p53-negative group.

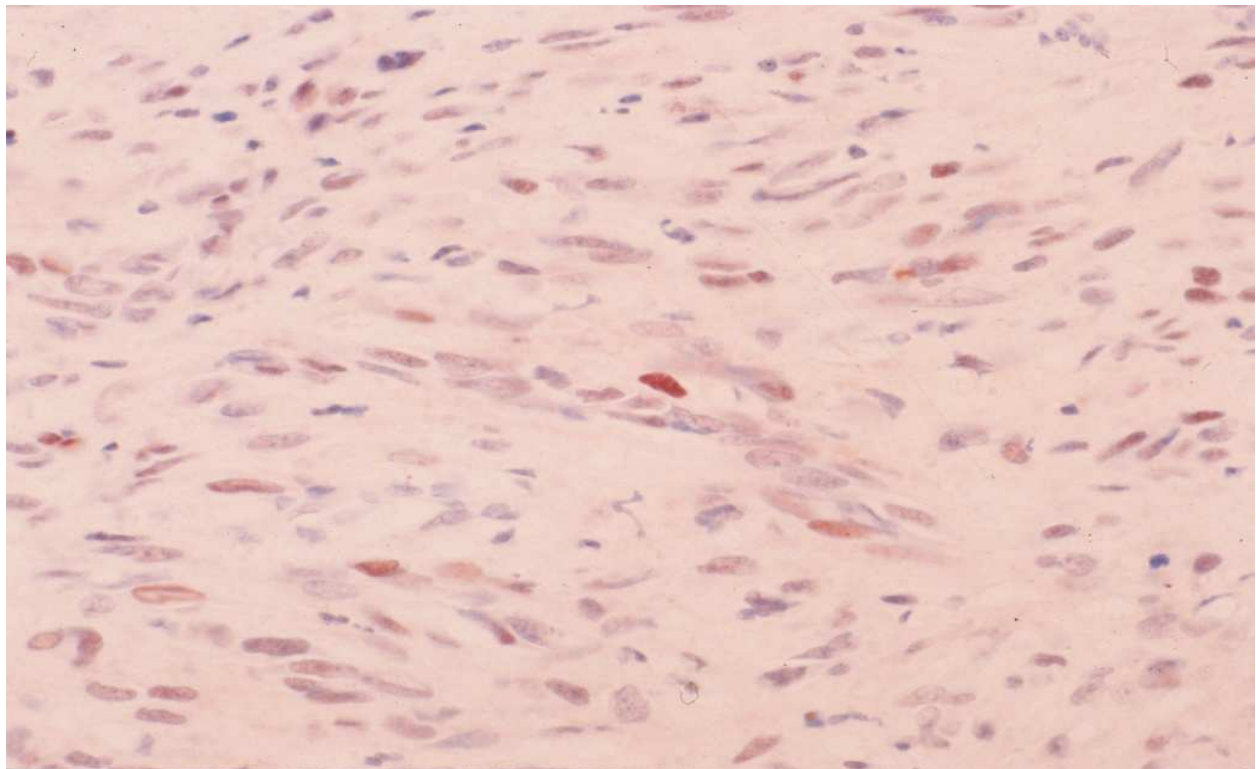


Fig. 2. p53 staining; Immunohistochemical staining with a monoclonal antibody against p53. Nuclear staining was observed in the tumor cells ($\times 400$).

The mean Ki-67 labeling index in the p53-positive group was 38.9 ± 8.26 and in the p53-negative group was 18.6 ± 14.6 . The mean Ki-67 labeling index in the p53-positive group was significantly higher than that in the p53-negative group ($p < 0.05$).

The p53-positive rate in the c-kit-positive group was 42.9% (three of seven), and in the c-kit-negative group was 20.0% (one of five), with no significant difference between them (Table 5).

Following DNA damage, p53 protein levels rise dramatically, and the entry into S is delayed until the genomic lesions are fully repaired. When the p53 function is lost, cells enter S without appropriate DNA repair, leading to fixation and propagation of genetic alterations. p53 overexpression promotes the transcription of p21, the product of which causes growth arrest through inhibition in Cdks, which are required for G1 to S transition. p21 is induced by DNA-damaging agents that trigger G1 arrest or apoptosis in cells with wild type p53 but not in tumor cells harboring a deletion or mutation in the p53 gene. Nikaido et al. reported that survival in gastric leiomyosarcoma of p53-positive cases was significantly shorter than that of p53-negative cases, that immunohistochemical p53 positivity was correlated with malignant behavior, and that p53 immunoreaction was a prognostic variable. In our cases, three cases of the metastasis/recurrence group were positive for p53 expression. The Ki-67 labeling index in all p53-positive cases was $>30\%$. There was no p53-positive case in the

benign group. The mean tumor diameter of the p53-positive cases was higher than that of the p53-negative cases. The mean Ki-67 labeling index of the p53-positive cases was higher than that of the p53-negative cases. The expression to p53 was considered to be a useful marker as an index of malignancy and prognosis in GIST.

4.4 bcl-2 expression

The immunoreactivity of bcl-2 was mainly identified in the cytoplasm of tumor cells (Fig. 3). Seven patients were positive for bcl-2 expression (Table 3).

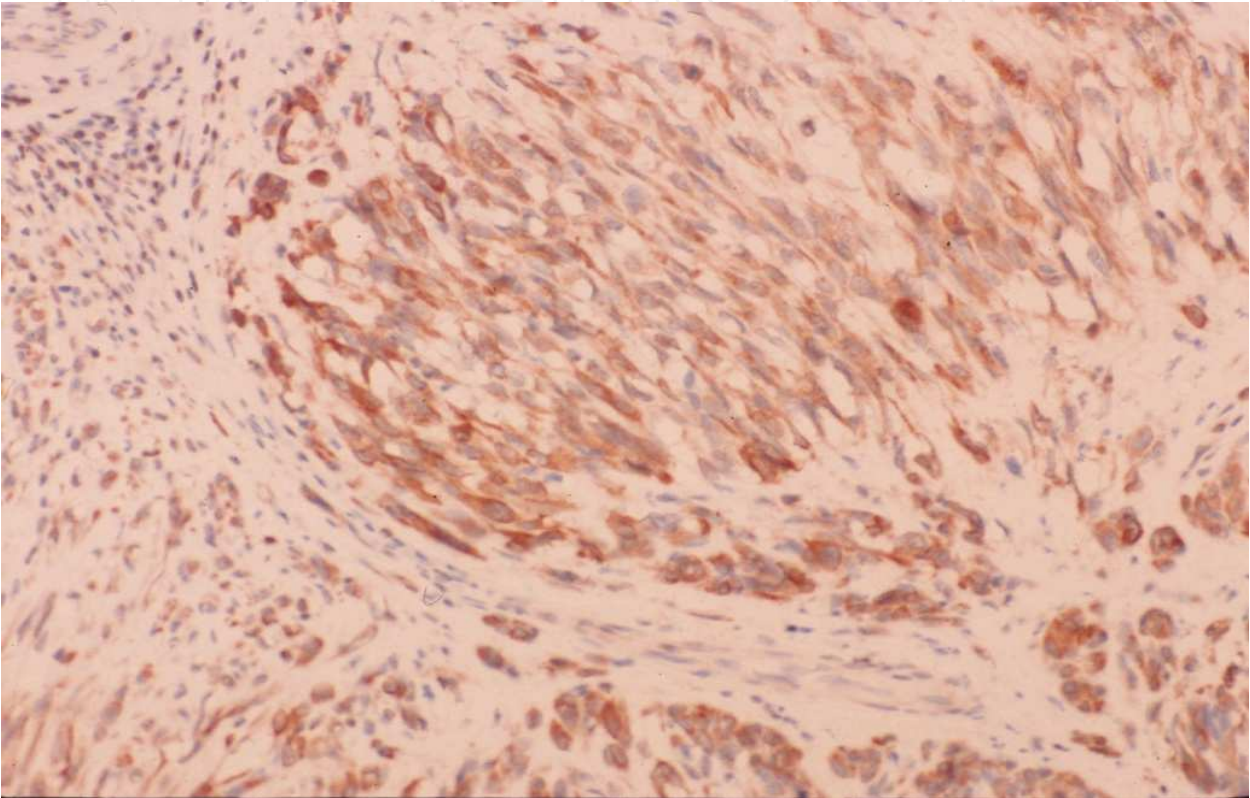


Fig. 3. bcl-2 staining; Immunohistochemical staining with a monoclonal antibody against bcl-2. Positive staining was observed in the cytoplasm of the tumor cells (×400).

All four cases of the metastasis/recurrence group were positive for bcl-2 expression (Table 3). The positive rate of bcl-2 in the malignant group was 60.0% (three of five), in the borderline group 50.0% (one of two) and in the benign group it was 60.0% (three of five; Table 4). The mean tumor size in the bcl-2-positive group was 77.1±64.4 mm, and in the bcl-2- negative group was 32.0±14.4 mm, with no significant difference between them.

The mean Ki-67 labeling index in the bcl-2-positive group was 28.3±15.8 mm, and in the bcl-2-negative group was 21.3±17.0 mm, with no significant difference between them.

The bcl-2-positive rate in the c-kit-positive group was 57.1% (four of seven), and in the c-kit-negative group was 60.0% (three of five), with no significant difference between them (Table 5).

The bcl-2-positive rate in the p53-positive group was 100% (four of four), and in the p53-negative group was 37.5% (three of eight), with no significant difference between them.

Cunningham et al. reported that the patients whose tumor demonstrated staining for bcl-2 protein had a shorter survival compared with those whose tumor did not demonstrate bcl-2. Noguchi et al. reported that overexpression in bcl-2 may play an important role in increasing the malignant potential, and furthermore, that the Ki-67 labeling index and bcl-2 overexpression may be useful in predicting malignant potential. In our study, all four cases of the metastasis/recurrence group were positive for bcl-2 expression, and all p53-positive cases were positive for bcl-2 expression. However, no significant correlation was observed between the frequency of the bcl-2 overexpression and the malignancy in the GIST, tumor diameter, or Ki-67 labeling index,

5. TUNEL staining

Positive staining was recognized in the nuclei in the apoptotic tumor cells (Fig. 4). The apoptotic count ranged between 0.1 to 3.9 (mean 1.20 ± 1.14 ; Table 3). The apoptotic count in the malignant group was 1.44 ± 1.65 , in the borderline group was 0.50 ± 0.57 , and in the benign group was 0.80 ± 0.71 , with no significant difference among them. The apoptotic count in the c-kit-positive group was 1.44 ± 1.32 , and in the c-kit-negative group was 0.88 ± 0.88 , with no significant difference between them (Table 5). The mean apoptotic count in the p53-positive group was 2.33 ± 1.20 , and in the p53-negative group was 0.65 ± 0.62 . The mean apoptotic count in the p53-positive group was higher than that in the p53-negative group. The mean apoptotic count in the bcl-2-positive group was 1.80 ± 1.16 , and in the bcl-2-negative group it was 0.38 ± 0.36 , with a significant difference ($p < 0.05$). All apoptotic counts of malignant group or metastasis/recurrence group were 1.0 or > 1.0 .

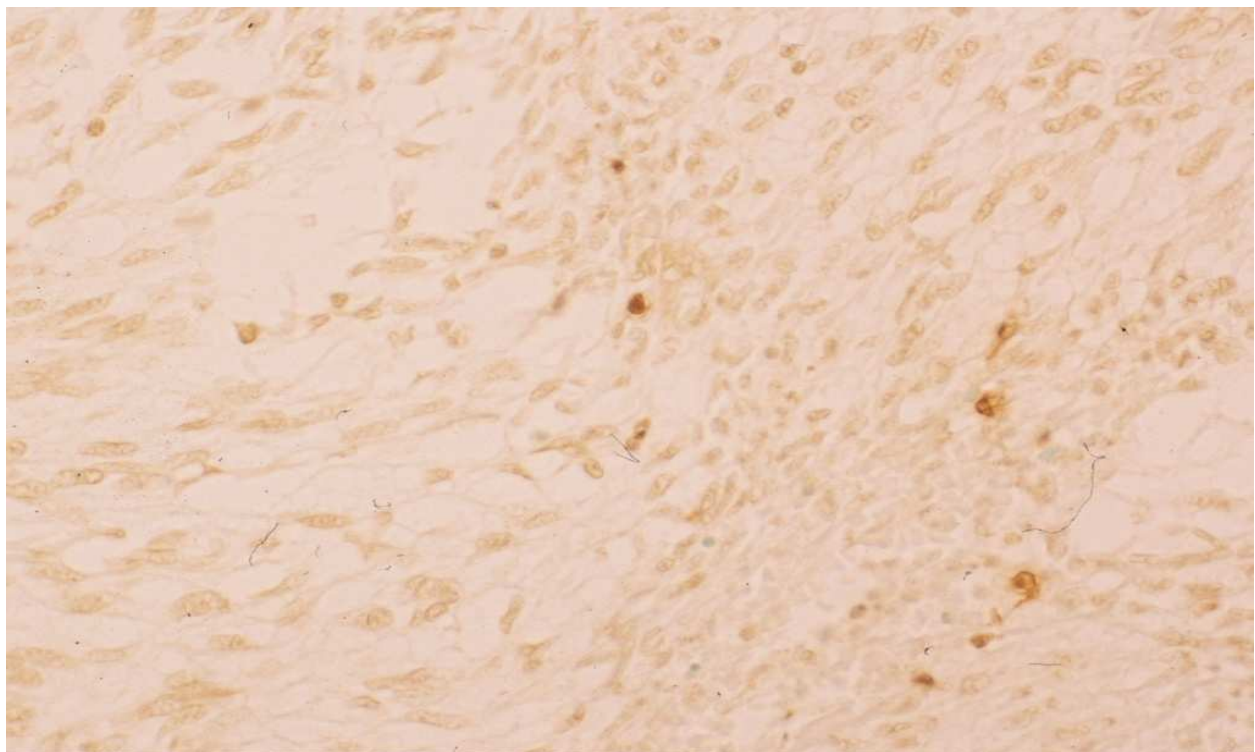


Fig. 4. TUNEL staining; TUNEL-positive staining was recognized in the nucleus of apoptotic tumor cells ($\times 200$).

| Case | c-kit | CD34 | Vimentin | α -SMA | Desmin | S-100 | p53 | bcl-2 | Ki-67 | Apoptosis |
|------|-------|------|----------|---------------|--------|-------|-----|-------|-------|-----------|
| 1 | + | + | + | - | - | - | + | + | 49.8 | 2.0 |
| 2 | + | + | + | + | - | - | - | - | 19.8 | 0.1 |
| 3 | + | - | + | + | - | - | - | - | 32.1 | 0.2 |
| 4 | + | + | + | - | - | - | + | + | 40.2 | 1.0 |
| 5 | + | + | + | - | - | - | - | - | 44.1 | 0.9 |
| 6 | - | + | + | - | - | - | + | + | 30.8 | 2.4 |
| 7 | - | + | + | - | - | - | - | + | 9.7 | 0.6 |
| 8 | - | - | + | - | - | - | - | - | 4.7 | 0.6 |
| 9 | + | + | + | - | - | - | - | + | 26.8 | 2.0 |
| 10 | - | - | + | - | - | - | - | + | 6.1 | 0.7 |
| 11 | - | + | + | - | - | - | - | - | 5.6 | 0.1 |
| 12 | + | + | + | - | - | - | + | + | 34.6 | 3.9 |

Table 3. Results from immunohistochemistry, Ki-67 index and the apoptotic count

| | Malignant (n=5) | Borderline (n=2) | Benign (n=5) |
|------------------------|--------------------|---------------------|-----------------|
| Ki-67 Index* | 35.3±11.0 | 37.5±9.40 | 10.6±9.26 |
| Apoptotic count | 1.44±1.65 | 0.50±0.57 | 0.80±0.71 |
| p53 positive cases | 3 (60.0%) | 1 (50.0%) | 0 |
| bcl-2 positive cases | 3 (60.0%) | 1 (50.0%) | 3 (60.0%) |
| c-kit positive cases** | 5 (100%) | 1 (50.0%) | 1 (20.0%) |

*. The Ki-67 Index in the malignant group was significantly higher than that in the benign group (p < 0.01).
**. The positive rate of c-kit in the malignant group was significantly higher than in the borderline and benign group (P < 0.05).

Table 4. Ki-67 index, Apoptotic Count and the expressions of p53, bcl-2 and c-kit according to Malignancy

Concerning apoptosis, Cunningham et al. reported that inhibition in apoptosis may be associated with malignant behavior in patients with gastrointestinal stromal/smooth muscle tumors. Yuki et al. reported that the apoptotic cell counts of leiomyosarcoma were significantly higher than those of leiomyoma, but this seems not to be a practical index for discrimination because apoptotic cell death is a rare event in gastrointestinal myogenic tumors. In our study, there was no significant difference in apoptotic count among the benign group, borderline group, and the malignant group. The mean apoptotic count in the bcl-2-positive group was higher than that in the bcl-2-negative group, and the mean apoptotic count in the p53-positive group was higher than that in the p53-negative group. p53 induces upregulation in bax protein which accelerates apoptotic death. bcl-2 protein is able to repress a number of apoptotic death programs. bax homodimerizes and forms heterodimers with bcl-2 in vivo. The ratio of bcl-2 to bax determines survival or death following an apoptotic stimulus. Therefore, if overexpressed bax countered the death

repressor activity of bcl-2, and apoptosis was considered to be induced in cases that were positive for bcl-2 expression.

| | c-kit-positive cases (n=7) | c-kit-negative cses (n=5) |
|----------------------|-------------------------------|------------------------------|
| Tumor size | 76.4±62.2 mm | 33.0±21.0 mm |
| Ki-67 Index* | 35.3±10.2 | 11.4±11.0 |
| Apoptotic count | 1.44±1.32 | 0.88±0.88 |
| p53-positive cases | 3 (42.9%) | 1 (20.0%) |
| bcl-2-positive cases | 4 (57.4%) | 3 (60.0%) |

*: Mean Ki-67 Index in the c-kit-positive cases was significantly higher than that in the c-kit-negative cases (p < 0.01).

Table 5. Tumor Size, Ki-67 index, apoptotic count, and the expressions of p53 and bcl-2 according to the expression of c-kit

6. Conclusion

Here, we report the Ki-67 labeling index, the expression of c-kit, p53, bcl-2, and apoptosis in eleven gastrointestinal stromal tumors (GISTs). The positive rate of c-kit in the malignant group was higher than in the borderline and benign group . The Ki-67 labeling index in the malignant GIST group was higher than that in the benign group. The Ki-67 labeling index in the c-kit-positive group was higher than that in the c-kit-negative group. The Ki-67 labeling index in the p53-positive cases was higher than that in the p53-negative cases. The bcl-2 expression was not correlated with potential malignancy. All metastasis/recurrence cases were bcl-2 positive. The apoptotic count in the bcl-2-positive cases was higher than that in the bcl-2-negative cases. All apoptotic counts of malignant group or metastasis/recurrence group were 1.0 or > 1.0. The high Ki-67 labeling index, the c-kit positive, the p53 over expression, the bcl-2 over expression and high apoptotic count were useful in predicting the potential malignancy of GIST.

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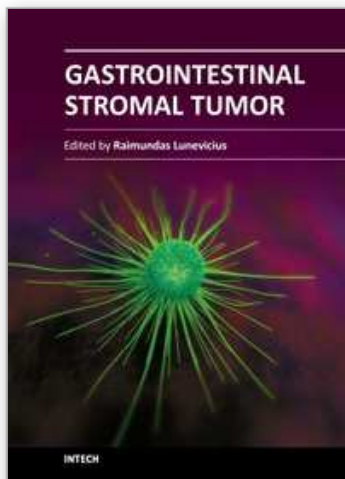
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Gastrointestinal Stromal Tumor

Edited by Prof. Raimundas Lunevicius

ISBN 978-953-51-0580-0

Hard cover, 120 pages

Publisher InTech

Published online 27, April, 2012

Published in print edition April, 2012

Almost 30 years have gone by since the postulation that GISTs derive from mesenchymal stem elements, and only 15 years have gone by since the definitive detection of origin of GISTs. Research in the last decade was more focused upon the justification of imatinib mezylate therapy in GISTs and clarification why a secondary resistance that occurred during the kinase inhibitors therapy. The era of therapy for GISTs, targeting the primary activating mutations in the KIT proto-oncogene; is being proclaimed as bringing the message of special importance to the pathologist role in multidisciplinary team that are responsible for treating patients with locally advanced or metastatic GIST. This is the first conclusive message forthcoming from this book. On the other hand, the book provides summarised and case-based knowledge on current management of gastrointestinal and extragastrointestinal stromal tumours. We hope that this book may be considered as a worthwhile timely addition to clinical science dissemination, medical education, further basic and clinical research.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Keishiro Aoyagi, Kikuo Kouhuji and Kazuo Shirouzu (2012). The Significance of the Ki-67 Labeling Index, the Expression of c-kit, p53, and bcl-2, and the Apoptotic Count on the Prognosis of Gastrointestinal Stromal Tumor, *Gastrointestinal Stromal Tumor*, Prof. Raimundas Lunevicius (Ed.), ISBN: 978-953-51-0580-0, InTech, Available from: <http://www.intechopen.com/books/gastrointestinal-stromal-tumor/prognosis-factor-of-gastrointestinal-stromal-tumor>

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