Ki-67 as a prognostic marker in colorectal cancer but not in gastric cancer

C.T.F. OSHIMA, K. IRIYA, N.M. FORONES

Oncology Group, e-mail: nora@gastro.epm.br, Gastroenterology Division and Pathology Department Universidade Federal de Sao Paulo, Brazil

Received January 26, 2005

The growth of tumors is highly variable and this probably reflects even its clinical course. The monoclonal antibody Ki-67 recognises an antigen present in the nuclei of cells in all phases of the cell cycle except G_0.

In the current study, we examined by immunohistochemistry the proliferative activity, based on Ki-67 labeling index (Ki67LI), in formalin-fixed and paraffin-embedded sections of 152 tumors, being 70 gastric and 89 colorectal cancers. The results obtained were correlated with the clinicopathologic factors.

The carcinomas showed a wide range of Ki-67LI, reflecting a variation in proliferative activity. The tumor labeling index ranged from 10 to 85 per cent positivity, being the mean level in gastric cancer tissue 0.52 and in colorectal cancer 0.44. There was also heterogeneity of labeling within many of the tumors. No significant correlation was found between Ki-67LI and sex, age, clinical stage in these cancers. In colorectal cancer, but not in gastric cancer, high levels of Ki67LI have been correlated with poor survival.

Ki-67 staining is a simple and useful method for estimating proliferative activity. The importance of Ki-67 as an indicator of tumor behaviour is not clear. In colorectal cancer this index may be used as a marker of prognosis.

Key words: Ki-67, immunohistochemistry, colorectal cancer, gastric cancer, cell proliferation

The growth of tumors is highly variable and this probably reflects even its clinical course. Methods of assessing cell proliferation in routinely fixed and processed tissues are of great interest in histopathology because they preserve tissue architecture and allow retrospective studies.

The classical morphological method for assessing cellular proliferation is the identification and counting of mitoses. Other methods are bromodeoxyuridine labeling [18], flow cytometry [14], nucleolar organizer regions [19] and immunohistochemistry [11, 12, 16].

Ki-67 is the monoclonal antibody that recognises an antigen present in the nuclei of cells in all phases of the cell cycle except G_0. A simple immunohistological method using this antibody allows the demonstration of proliferating cells [11, 12].

Recent reports have described the immunohistological reactivity of Ki-67 in colorectal and gastric cancers, and they have suggested that Ki-67 immunostaining may provide data of clinical value [2, 8, 20].

Gastric cancer is the fourth most common, and colorectal cancer the third most common cancer in the world [10]. In Brazil 23160 new cases of gastric cancer and of colorectal cancer had been diagnosed in 2003 [3].

Although the surgical resection of the tumor is possible, the recurrence of the cancer is the main cause of death among these patients. Until now, pathologic variables associated with clinical findings have been the most important form to establish the prognosis. These parameters, however, do not reflect biologic behaviour of the individual cancer tissue that correlates with tumor aggressiveness and reccurrence risk, which may enable some groups to undergo a more intensive adjuvant treatment. In recent years, the results of research aims to identify the correlation between cellular proliferative activity and the malignant potential of recurrence derived from various methods of cell proliferation have been conflicting.

The purpose of this study was to investigate the proliferative activity by studying the Ki-67 immnunostaining in colorectal and gastric adenocarcinomas as well as to correlate this activity with prognosis.

Patients and methods

Retrospectively, 70 cases of gastric adenocarcinomas and 89 cases of colorectal adenocarcinomas were analyzed using paraffin-embedded blocks. The cancer tissues were fixed in formalin and routinely developed by using the paraffin-embedding method, thus obtaining 3 μm-thick tissue sections. The pathologist revised all the hematoxilin-eosin stained slides and the diagnosis was confirmed.

The patients were staged according to the UICC classification, and gastric cancer was also classified into intestinal or diffuse according to the classification of Lauren.

Immunohistochemistry. Sections of 3 μm were dewaxed in xylene, taken through ethanol to water to rehydrate and submitted to microwave irradiation for 15 min in 10 mM citrate buffer (pH 6.0) to detect masked and unmasked antigen sites.

Endogenous peroxidase activity was blocked by incubating the sections in a solution of 3% hydrogen peroxide in phosphate buffered saline (PBS) for 20 minutes. After washing in PBS, the sections were incubated with the primary monoclonal antibody Ki-67, clone MIB-1 (DAKO) in the dilution of 1:100, overnight at 4 °C. The sections were washed with PBS and incubated with a biotinylated secondary antibody for 30 minutes, followed by incubation with streptoavidine-biotin-peroxidase complex for further 30 minutes, at room temperature. Staining was carried out using a solution 3-3'diaminobenzidine (Sigma), containing 1% hydrogen peroxide. Finally, sections were lightly counterstained using Harris hematoxilin.

Sections were scored as positive if a distinct nuclear immunoreaction was found in the identifiable tumor cells. A section of colorectal and gastric adenocarcinoma with intense Ki-67 expression was included as a positive control.

The Ki-67 staining intensity and patterns were analysed by two observers who were unaware of patient identity. Any scoring discrepancies were resolved by consensus. The labeling index was determined observing 1000 nuclei in areas of the section with the highest labeling rates, and the percentage of Ki-67-labeled nuclei was used for analyses. The tumors were also classified into two groups, as follows: A <40% and B ≥40%.

Statistical analysis. The clinical stages were categorized in fewer categories (I+II and III+IV) to avoid statistics with small numbers. The Mann-Withney test was used for comparison of continuous variables. The calculus of the survival estimators was carried out through the Kaplan-Meier technique. The Cox's proportional hazards model was used for multivariate analysis. A level of 5% significance was adopted. Descriptive levels (p) below this value were considered significant.

Table 1. Clinical parameters of patients with gastric or colorectal cancer

Clinical parameters of the patients		GC N (%)	CRC N (%)
Sex	Male	47 (67)	36 (40)
	Female	23 (33)	53 (60)
Age	< 50	14 (20)	19 (23)
	≥ 50	56 (80)	64 (77)
Stage	I	19 (27)	0
	II	15 (22)	42 (47)
	III	22 (32)	40 (45)
	IV	12 (17)	7 (8)
Follow-up	Alive	28 (40)	50 (56)
	Recurrence	4 (6)	2 (2)
	Death due to the cancer	24 (34)	25 (28)
	Death due to another cause	6 (9)	0
	Lost to follow up	8 (11)	12 (14)

N – number of patients, GC – gastric cancer, CRC – colorectal cancer

Results

The clinical parameters of patients with colorectal and gastric cancer were described in Table 1. The photomicrography of proliferative activity of gastric and colorectal adenocarcinoma as defined by Ki-67 immunohistochemistry was presented in Figure 1.

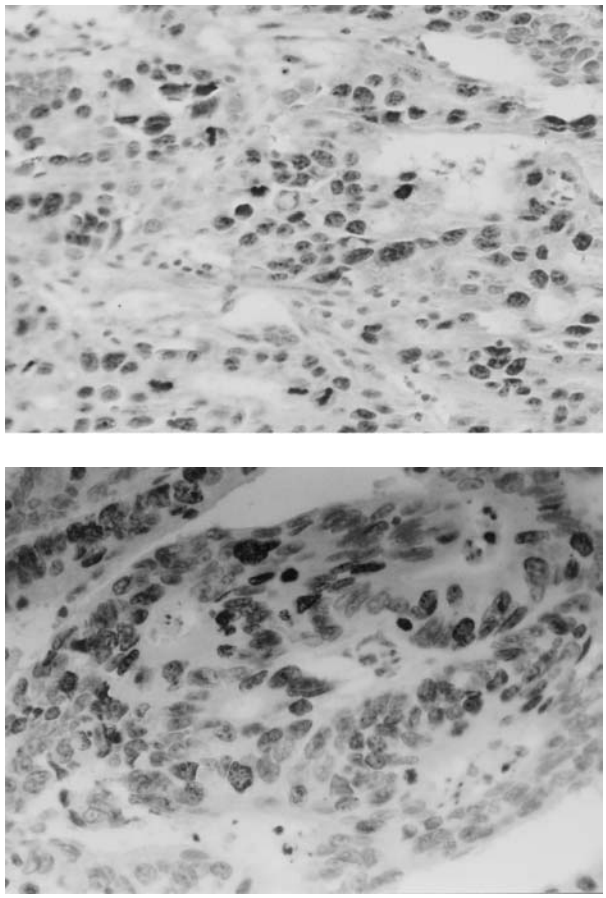

Figure 1. Photomicrography showing proliferative activity in gastric (A) and colorectal adenocarcinomas (B) as defined by Ki-67 immunohistochemistry.

In the gastric cancer group 47 patients were male and 23 female, the mean age was 62±14 years. In relation to the clinical stage, 49% were stage I and II; 49% were stages III and IV and in 2% of the cases it was not possible to determine the clinical stage. The tumors were most frequently located in the antrum (63%). According to Lauren classification 48 (60%) were of the intestinal type. The patients were observed for a period of time that varied from one to 84 months (average of 33 months). On the time of the study, 46% remained alive (40% without disease). The mean level of Ki67LI was 0.52±0.18.

In the colorectal cancer group 36 patients were male and 53 female. The mean age of colorectal cancer patients was 59±14 years, 47% were stage II, 45% stage III and 8% IV. Thirty-three had cancer in the rectum and 56 in the colon. The patients were observed for a period of time that varied from 10 to 84 months (average of 42 months). On the time of the study, 58% remained alive (56% without disease). The mean level of Ki67LI was 0.44±0.16.

Ki-67 immunoreactivity was found in the nuclei of neoplasic cells in all cases of gastric and colorectal cancers (Fig. 1). In general, diffuse nuclear staining was present in all cases and there were however intra-tumor variations in the distribution of labeled cells.

We did not observe any correlation between Ki67LI and sex, age, clinical stage in either cancer (Tab. 2 and 3). There was no difference in the Ki67LI between the intestinal and diffuse type (p=0.41). There was no difference in the Ki67LI in colon cancers when compared to rectal cancer (p=0.33). Kaplan-Meier's curve of survival was not different for group A (<40%) or B (≥40%) in gastric or colorectal cancer (Fig. 2 and 3). Cox regression showed that Ki67 is an independent predictor of survival in colorectal cancer (p=0.009), but not in gastric cancer (p=0.44) (Tab. 4).

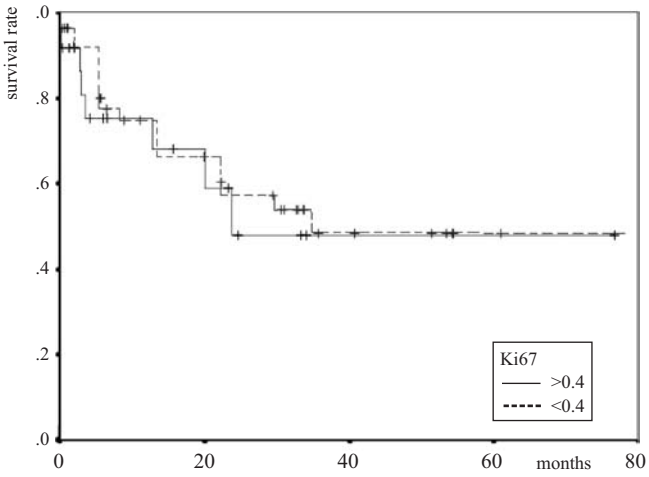

Figure 2. Survival of patients according to the values of Ki-67LI <0.40 or ≥0.40 in gastric cancer.

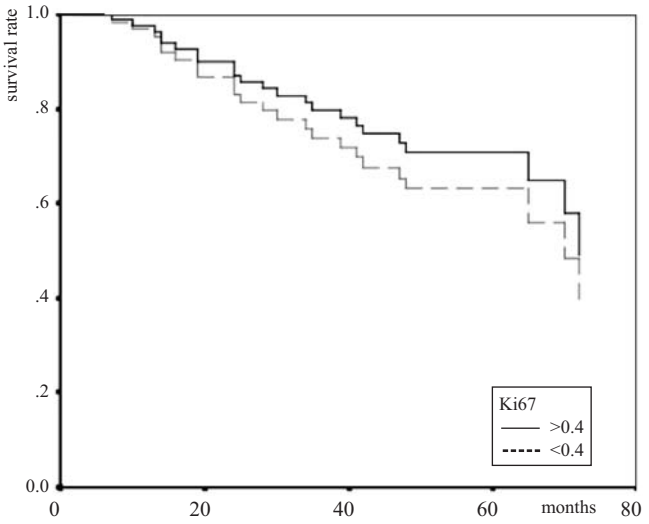

Figure 3. Survival of patients according to the values of Ki-67LI <0.40 or ≥0.40 in colorectal cancer.

Discussion

Previous studies have shown conflictive results about the correlation between proliferative activities and worse prognosis in a variety of tumors. As growth fraction is one of the important variables in tumor cell kinetics, it was chosen to be investigated.

Immunohistochemical methods of assessing cell proliferation have particular advantages over other techniques because of the maintenance of cellular and tissue architecture, the relative simplicity of the methodology, and the rapidity of results [13].

The suitability of monoclonal antibody Ki-67 for detection of growth fraction in tumors had previously been reported in studies in malignant lymphoma [10] and breast tumor [23]. Besides these results, the usefulness of the Ki-67LI for predicting tumor progression and prognosis in patients with colorectal and gastric cancer has been reported [1, 2, 15].

Ki-67LI ranged from 0.10 to 0.82 with a mean of 0.44 in the cases of colorectal carcinoma, and from 0.13 to 0.86 with a mean of 0.52 in gastric carcinoma, which was significantly' higher than for colorectal carcinoma.

In a study published by the same authors in 38 colorectal cancers tissue had shown a similar mean of Ki67LI (0, 40). However, there were no differences among Dukes' stage, percentual of recurrence and this index. Due to the small number of tumors the results had not been compared with survival [8].

KAKEJI et al [17] have shown that the combination analysis of depth of invasion with Ki-67 LI gives a more precise prediction of nodal metastasis, compared with histological analysis alone in gastric cancer. We did not find this correlation. Although a previous study [20] reported a strong suggestion that diffuse carcinomas had a tendency to have higher

Table 2. Mean levels (SD) of Ki67LI and clinical parameters of patients with gastric cancer

Clinical parameters of the patients		GC Mean (SD)	p
Sex	Male	56.80 (14.00)	1.33
	Female	50.70 (18.49)	
Age	< 50	53.00 (20.61)	0.83
	≥ 50	51.61 (17.16)	
Stage	I+II	48.86 (20.10)	0.98
	III+IV	51.82 (16.66)	
Lauren Classification	Diffuse	0.55 (0.17)	0.41
	Intestinal	0.49 (0.20)	

Table 3. Mean levels (SD) of Ki67LI and clinical parameters of patients with colorectal cancer

Clinical parameters of the patients		Mean (SD)	p
Sex	Male	0.47 (0.21)	0.42
	Female	0.43 (0.15)	
Age	< 50	0.44 (0.20)	0.87
	≥ 50	0.43 (0.16)	
Stage	I+II	0.43 (0.17)	0.34
	III+IV	0.39 (0.18)	
Local	Colon	0.41 (0.16)	0.33
	Rectum	0.45 (0.18)	

Table 4. The Cox's proportions hazards model on gastric and colorectal cancer

	B	SE	Df	Sig
GC	-0.09	0.012	1	0.442
CRC	0.239	0.092	1	0.009

SE – standard error, Sig – significance, GC – gastric cancer, CRC – colorectal cancer

Ki-67LI as compared to intestinal carcinomas, in this study the differences was not statistically different.

We did not find any correlation between the Ki-67LI and sex, age, histological type, or clinical stage of patients in colorectal and gastric cancer.

High proliferating activity of tumors was associated with poor survival of patients in colorectal cancer but we have been unable to demonstrate the same correlation in gastric cancer.

Several studies measuring proliferative activity in cancer Dukes' stage B2 or C using flow cytometry noted that a high proliferative index predicts great probability of recurrence, and diminished survival [5, 24].

Besides the correlation between proliferative activity and survival, some assays on flow cytometric DNA analysis suggest that highly proliferative tumors have an increased sensitivity to neoadjuvant and adjuvant chemotherapy in breast cancer [21, 22]. In contrast, the chemotherapy for slowly proliferating tumors is controversial [4, 6]. Colorectal cancer with a high proliferative activity is more likely to respond to radiotherapy [9] and chemotherapy [1, 2].

According to these results, proliferative markers have the potential to distinguish patients with rapidly proliferating tumors that are likely to respond to chemotherapy from patients with slowly proliferating tumors who may not need aggressive treatment. Selection of those patients, by assessment of the proliferative activity, may be a reliable approach to predict which patients will respond to chemotherapy.

References

[1] ALLEGRA CJ, PAIK S, COLANGELO LH, PARR AL, KIRSCH I et al. Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes'B and C colon cancer: a National Cancer Institute-National Surgical Adjuvant breast and bowel project collaborative study. J Clin Oncol 2003; 21: 241–250.

[2] ALLEGRA CJ, PARR AL, WOLD LE, MAHONEY MR, SARGENT DJ et al. Investigation of the prognostic and predictive value of thymidylate synthase, p53, and Ki-67 in patients with locally advanced colon cancer. J Clin Oncol 2002; 20: 1735–1743.

[3] Brasil, Ministério da Saúde, Instituto Nacional do Cancer. Estimativa da incidencia e mortalidade por cancer no Brasil, www.http.INCA.org.br 2003.

[4] BROET P, ROMAIN S, DAVER A, RICOLLEAU G, QUILLIEN V et al. Thymidine kinase as a proliferative marker: clinical relevance in 1,692 primary breast cancer patients. J Clin Oncol 2001; 19: 2778–2787.

[5] CASCINU S, LIGI M, GRAZIANO F, DEL FERRO E, VALENTINI M et al. S-phase fraction can predict event free survival in patients with pT2-T3N0M0 colorectal carcinoma: Implications for adjuvant chemotherapy. Cancer 1998; 83: 1081–1085.

[6] CHASSEVENT A, JOURDAN ML, ROMAIN S, DESCOTES F, COLONNA M et al. S-phase fraction and DNA ploidy in 633 T1T2 breast cancers: a standardized flow cytometric study. Clin Cancer Res 2001; 7: 909–917.

[7] FERLAY J, BRAY P, PISANI P, PARKIN DM. Cancer incidence, mortality and prevalence worlwide. International Agency of Research in Cancer. World Health organization. IARC Press, Lyon 2001.

[8] FORONES NM, OSHIMA C, NANOGAKI S, TANAKA M, BARBOSA V. Determination of proliferative activity using Ki67 and expression of p53 in colorectal cancer. Arq Gastroenterol 1999; 36(3): 122–126.

[9] FRINDEL E, TUBIANA M. Radiobiology and the cell cycle. In: Baserga R, editor. The Cell Cycle and Cancer. New York: Marcel Dekker, 1971: 389–447.

[10] GERDES J, DALLENBACH F, LENNERT K. Growth fractions in malignant non-Hodgkin's lymphoma (NHL) as determined in situ with the monoclonal antibody Ki-67. Hematol Oncol 1984; 2: 365–371.

[11] GERDES J, LEMKE H, BAISCH H, WACKER H-H, SCHWAB U, STEIN H. Cell cycle analysis of a proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunology 1984; 133: 1710–1715.

[12] GERDES J, SCHWAB U, LEMKE H, STEIN H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983; 31: 13–20.

[13] HALL PA, LEVISON DA. Assessment of cell proliferation in histological material. J Clin Pathol 1990; 43: 184–192.

[14] HEDLEY DW, FRIEDLANDER ML, TAYLOR LW, RUGG CA, MUSGROVE EA. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 1983; 31: 1333–1335.

[15] IGARASHI N, TAKAHASHI M, OHKUBO H, OMATA K, IIDA R, FUJIMOTO S. Pedictive value of Ki-67, p53 protein, and DNA content in the diagnosis of gastric carcinoma. Cancer 1999; 86: 449–454.

[16] IRAZUSTA SP, VASSALO J, MAGNA LA, METZE K, TREVISAN M. The value of PCNA and AgNOR staining in endoscopic biopsies of gastric mucosa. Pathol Res Pract 1998; 194: 33–39.

[17] KAKEJI Y, KORENAGA D, TSUJITANI S, HARAGUCHI M, MAEHARA Y, SUGIMACHI K. Predictive value of Ki-67 and argyrophilic nucleolar organizer region staining for lymph node metastasis in gastric cancer. Cancer Res 1991; 51: 3503–3506.

[18] OHYAMA S, YONEMURA Y, MIYAZAKI I. Prognostic value of S-phase fraction and DNA ploidy studied with in vivo administration of bromodeoxyuridine on human gastric cancers. Cancer 1990; 65: 116–121.

[19] OSHIMA CTF, FORONES NM. AgNOR in stomach neoplasm. Arq Gastroenterol 2001; 38(2): 89–93.

[20] PRAKASH I, MATHUR RP, KAR P, RANGA S, TALIB VH. Comparative evaluation of cell proliferative indices and epidermal growth factor receptor expression in gastric carcinoma. Indian J Pathol Microbiol 1997; 40(4): 481–490.

[21] SIMPSON JF, GRAY R, DRESSLER LG, COBAN CD, FALKSON CI et al. Prognostic value of histologic grade and proliferative activity in axillary node-positive breast cancer: results from the Eastern Cooperative Oncology Group Companion Study, EST 4189. J Clin Oncol 2000; 18: 2059–2069.

[22] SPYRATOS F, BRIFFOD M, TUBIANA-HULIN M, ANDRIEU C, MAYRAS C et al. Sequential cytopunctures during preoperative chemotherapy for primary breast carcinoma. II. DNA flow cytometry changes during chemotherapy, tumor regression, and short-term follow-up. Cancer 1992; 69: 470–475.

[23] SPYRATOS F, FERRERO-POUS M, TRASSARD M, HACENE K, PHILLIPS E et al. Correlation between MIB-1 and other proliferation markers. Cancer 2002; 94: 2151–2159.

[24] VENKATESH KS, WEINGART DJ, RAMANUJAM PJ. Comparison of double and single parameters in DNA analysis for staging and as a prognostic indicator in patients with colon and rectal cacinoma. Dis Colon Rectum 1994: 37: 1142–1147.