ANTICANCER RESEARCH *25*: 3083-3088 (2005)

Nuclear Area is a Prognostic Determinant in Advanced Colorectal Cancer

ABDELBASET BUHMEIDA[1,3], RAIJA RISTAMÄKI[1], HANAN LAMLUM[1,3], RIYAD BENDARDAF[1,3], SUVI HEIKKILÄ[1], YRJÖ COLLAN[2], KARI SYRJÄNEN[1] and SEPPO PYRHÖNEN[1]

Departments of [1]Oncology and Radiotherapy, and [2]Pathology, Turku University Central Hospital, and [3]MediCity Research Laboratory, University of Turku, Turku, Finland

Abstract. *Background: The prognostic value of morphometric nuclear features in Dukes' Stages B/C and D colorectal cancer (CRC) was assessed. Patients and Methods: Primary tumours from 86 CRC patients were analysed, using an image overlay drawing system (Prodit Morphometry Program), for the following nuclear features: area, perimeter, diameter, form factor, roundness. Results: The median nuclear area (NA) was 104.6 μm^2 (range 57.2 – 237.2 μm^2). The NA was larger in patients with lymph node metastasis ($p<0.02$). Altogether, 43% of the patients showed clinical response to irinotecan-based chemotherapy. All six patients with complete response (CR) had a NA above the median ($p<0.03$). The disease-specific survival of the patients with a NA above the median was significantly better than in patients with smaller NA ($p<0.02$). Conclusion: Using the median NA as the cut-off value seems to effectively discriminate patients who are likely to respond to irinotecan-based chemotherapy (with improved prognosis) from those who are non-responsive and develop progressive disease.*

Colorectal cancer (CRC) is one of the most common malignant tumours worldwide (1, 2), and its incidence rises with increasing age (3, 4). The prognosis of CRC has improved in recent decades (5, 6), partly based on advances in diagnosis and new therapeutic tools (7, 8). However, there is an urgent need for more specific molecular (or other) markers that could more accurately predict the disease outcome, or aid in selecting patients among special sub-groups for appropriate treatment, *i.e.*, to predict the response to treatment (9-11). Many characterised pathological and clinical variables are known to be associated with survival of CRC patients, but the individual prediction of disease outcome after curative resection is still unreliable (12).

During the past decades, a substantial effort has been made to test a wide variety of molecular and other markers to improve this prediction (13). Apart from molecular markers analysed using immunohistochemistry, morphological methods evaluating, *e.g.* nuclear profiles in cancer cells, have also been reported to provide useful prognostic and predictive information in various human cancers. Thus, changes in nuclear morphometric parameters have been shown to be of prognostic value, *e.g.* in breast (14, 15), pancreas (16), thyroid (17) and prostate cancer (18). The evident advantage of computerised morphometry lies in the fact that objective data are quickly obtained by using conventional microscopic analysis.

Despite the relative technical simplicity, computer-assisted image analysis of nuclear features has only rarely been applied in the study of colorectal adenocarcinomas (19). Some investigators have used morphometric measurements to analyse differences in nuclear size and shape of normal and neoplastic colorectal tissue (20). Not unexpectedly, the nucleus is usually larger and its shape is more often irregular in cancer cells. Until now, however, the value of nuclear morphometry in prediction and prognostication of CRC has not been comprehensively assessed (21, 22). In this work, a study on the prognostic value of the nuclear size in patients with advanced CRC, who were treated with irinotecan-based chemotherapy regimens, is presented.

Patients and Methods

Patients, treatment and follow-up. The material of the present study consisted of a series of 86 patients with advanced (Dukes' Stages B/C and D) colorectal carcinoma. All patients were diagnosed and treated at the Department of Oncology and Radiotherapy, Turku

Correspondence to: Abdelbaset Buhmeida, MD, Dr. Med. Sci., Department of Oncology and Radiotherapy, Turku University Central Hospital, P.O. Box 52 (Savitehtaankatu 1), FIN-20521 Turku, Finland. Tel: +358 2 3337016, Fax: +358 2 333 7000, e-mail: abuhme@utu.fi

Key Words: Nuclear morphometry, nuclear area, colorectal cancer, prognosis, adjuvant therapy.

Table I. *Patient and tumour characteristics at diagnosis.*

Variable	no. or value	(%)
Patients	86	
Male	54	63
Female	32	37
Age (yrs)		
Mean (range)	58.6 (24-80)	
Tumour localisation		
Rectum	21	24
Left colon	35	41
Right colon	23	27
Transversum	7	8
Dukes' Stage		
Stages B/C	31	36
Stage D	55	64
Primary nodal status		
N0	23	27
N+	44	51
Nx*	19	22
Histological grade		
G I	11	13
G II	57	66
G III	15	17
G x**	3	3
Metastasis at diagnosis		
M0	31	36
M1	55	64
Status at study end-point		
Alive without cancer	3	4
Alive with cancer	9	10
Dead as a result of cancer	73	85
Dead from other cause(s)	1	1
Site of metastasis		
Liver only	36	42
Lung only	5	6
Multiple sites	41	48
Locally advanced	4	
Time to develop metastasis (mo.)		
Mean (range)	9 (0-142)	
Duration of treatment (mo.)		
Median (range)	6 (1-18)	
Follow-up time (mo.)		
Median (range)	27.5 (5-150)	

* Patients who already had metastasis at diagnosis.
** Grade is not known

University Central Hospital, Turku, Finland, between October 1998 and August 2003. The key clinical characteristics of the patients are given in Table I. Of the 86 cases, 32 were women and 54 were men, with a mean age of 58.6 years (range 24-80). Altogether, 31 patients had Stages B/C and 55 had Stage D disease. As a part of their routine laboratory tests, serum levels of carcinoembryonic antigen (CEA) were measured at the time of diagnosis and when metastases appeared. The mean CEA values were 753 (SD 3.373) and 607 (SD 2.752), respectively.

All 86 patients were treated with chemotherapy: 20 were treated with irinotecan alone and 66 received a combination of irinotecan and 5-fluorouracil (5-FU). Irinotecan (350 mg/m^2) was administered as a 60 to 90-min intravenous *(i.v.)* infusion every 3 weeks. In the combination regimen, irinotecan (180-210 mg/m^2) was administered as 60 to 90-min intravenous infusion and 5-FU (500 mg/m^2, *i.v.* bolus) modulated with folinic acid (FA) (60 mg/m^2, *i.v.* bolus). The 5-FU/FA administrations were repeated again on the following day. The cycle was repeated every 2 weeks. The described treatment was continued until disease progression, or occurrence of unacceptable toxicity (23).

The clinical responses were monitored at clinical visits at 8- to 12-week intervals, and classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD), according to the WHO criteria (24). The follow-up data are available until the end of January 2005, with a mean follow-up time of 33.1 months (median 27.5 mo.). The general outcome was classified as: a) being alive without disease (n=3), b) alive with disease (n=9), c) died of disease (n=73) and d) died of other causes (n=1).

Morphometry. All samples were obtained from the primary tumour at the time of diagnosis. Histological samples were fixed in buffered formalin and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin. The nuclear profile of cancer nuclei was measured using the Prodit morphometry program (Prodit 3.1, Promis Inc, Almere, Netherlands), which is a digitising interactive image overlay system. The system includes a microscope, a personal computer (MultiSync 3D Colour Monitor; NEC, Japan), a video camera (JVC TK-870U; JVC Japan) and a digitiser board (PIP 512B video digitiser board; Matrox Electronic Systems, Dorval, Quebec, Canada). Analogue images of the nuclei were outlined on the monitor screen using a computer mouse, resulting in a digitised overlay of the traced outline.

The instrument was calibrated with a micrometer slide before each measurement. Measurements were carried out at X2,500 magnification on the monitor screen (X40 objective, X10 video ocular and X2 internal magnification). When examining the sections, the tumour cells from the most cellular area, often at the periphery of the tumour, were sought. Necrotic and inflammatory areas were discarded. An average of 10-15 microscopic fields was screened, and 100 consecutive tumour cells with clear nuclear borders were outlined and measured. Overlapping nuclei were not measured. Of the morphometric variables measured by the Prodit program, the nuclear area (NA) perimeter and diameter were assessed in this study (25).

Statistical analysis. Statistical analyses were performed using the SPSS® (SPSS, Inc., Chicago, IL, USA) and STATA (Stata Corp., Texas, USA) software packages (SPSS for Windows, version 12.0.1 and STATA/SE 8.2). To test association between NA and different biological and clinical parameters, frequency tables were analysed using the Chi-square test, with likelihood ratio (LR) or Fischer's exact test to assess the significance of the correlation between the categorical variables. Differences in the means of continuous

Table II. *Response to different treatment regimens.*

Response to treatment	No. of cases	Single[1]	Combination[2]
Complete response	6	0	6
Partial response	30	3	27
Stable disease	33	7	26
Progressive disease	15	9	6
Total[3]	84	19	65

[1]Irinotecan only
[2]Irinotecan, 5-FU, and folinic acid
[3]Response is not available in 2 cases.

variables were analysed using non-parametric tests (Mann-Whitney) or ANOVA (analysis of variance). Univariate survival (life-table) analysis for the outcome measure (disease-specific survival, DSS) was based on Kaplan-Meier method. Multivariate survival analysis was run by using Cox's proportional hazards model to analyse the independent prognostic predictors. In all tests, the values $p<0.05$ were regarded as statistically significant.

Results

The median NA analysed in the cancer samples was 104.6 μm^2 (range 57.2 – 237.2 μm^2) and was used as the cut-off to dichotomise the NA for calculating its correlations with the other parameters. The NA of tumour cells was clearly larger in patients who had lymph node (LN) metastases at diagnosis ($p<0.02$). However, there was no association between NA and the presence of distant (organ) metastases at diagnosis ($p=0.3$). The median NA of LN-positive patients was 111 μm^2 as compared to 98 μm^2 in LN-negative cases. Higher than median (16.8 μg/l, range 0.1-19.5 μg/l) serum carcinoembryonic antigen (s-CEA) level at diagnosis was significantly associated with smaller NA in cancer cells ($p<0.04$). Also s-CEA at metastasis correlated with NA, probably because 43/72 patients had metastasis at diagnosis.

No statistically significant correlations were found between the morphometric features and the clinico-pathological variables, including age, sex or histological grade of the tumour ($p=0.6$, $p=0.8$ and $p=0.7$, respectively).

The response to different treatment regimens is summarised in Table II. Forty-three percent (36/84) of the patients showed an objective response (6 CR, 30 PR) to irinotecan-based chemotherapy as the treatment for metastatic disease. Interestingly, all 6 patients who demonstrated CR had tumours with NA values above the median ($p<0.03$). In general, more patients showed a response to combination therapy (n=65), than did those treated with single irinotecan therapy only ($p=0.004$). Other clinico-pathological factors were not associated with the clinical response to treatment.

Univariate survival (Kaplan-Meier) analysis was used to test the influence of NA and clinico-pathological variables on survival. In all continuous variables, the median values were used as cut-off points in this model. Importantly, the disease-specific survival was significantly longer ($p<0.02$) in patients with NA values above the median, as compared with those who had lower NA values, as shown in Figure 1. Other predictors of favourable prognosis included low s-CEA-levels ($p=0.003$) and absence of distant metastases ($p<0.0001$) at the time of diagnosis.

A multivariate survival (Cox) analysis was performed to disclose the independent prognostic factors, by entering in the model all the studied variables. In the final model, the only factors with independent prognostic value were NA ($p=0.03$) (HR, hazard ratio=0.5, 95%CI, confidence level 0.3–0.9) and the presence of metastases at diagnoses ($p=0.008$) (HR=2.7, 95% CI 1.3–5.8).

Discussion

Computerised image analysis allows accurate and objective measurement of several nuclear features, and this technique has been used to demonstrate that increases in nuclear size and irregularity in their shape are more frequent in cancer cells than in borderline lesions (26, 27). Moreover, increasing abnormalities in nuclear morphology seem to parallel with tumour progression in various cancers, including renal cell carcinoma (28), breast cancer (29) and thyroid tumours (30).

Although the biological phenomena and mechanisms responsible for these nuclear alterations in tumour cells remain to be revealed, our present study clearly showed a close correlation between NA and lymph node metastasis and disease outcome in advanced CRC. Such an association with lymph node metastasis was described previously by Ikeguchi *et al.* (31), who found a larger mean NA in CRC patients with lymph node metastasis. In another study, the mean NA was significantly associated with the number of involved lymph nodes (32). Interestingly, Hamilton *et al.* (33) reported that gastric cancer patients with lymphatic invasion showed a large NA in tumour cells. In addition, Ikeguchi *et al.* (19) found that venous invasion and synchronous haematogenic metastasis in CRC were significantly more frequent among patients who had tumours with large NA, as compared to patients with small NA in their tumours.

Importantly, the present study showed that disease-specific survival was significantly better in patients with a NA above the median values. These data confirm the previous observations of Carson *et al.* (34), who reported a longer disease-free survival (DFS) in CRC patients with larger nuclear volume of their cancer cells as compared with those who died of their disease. However, contradictory

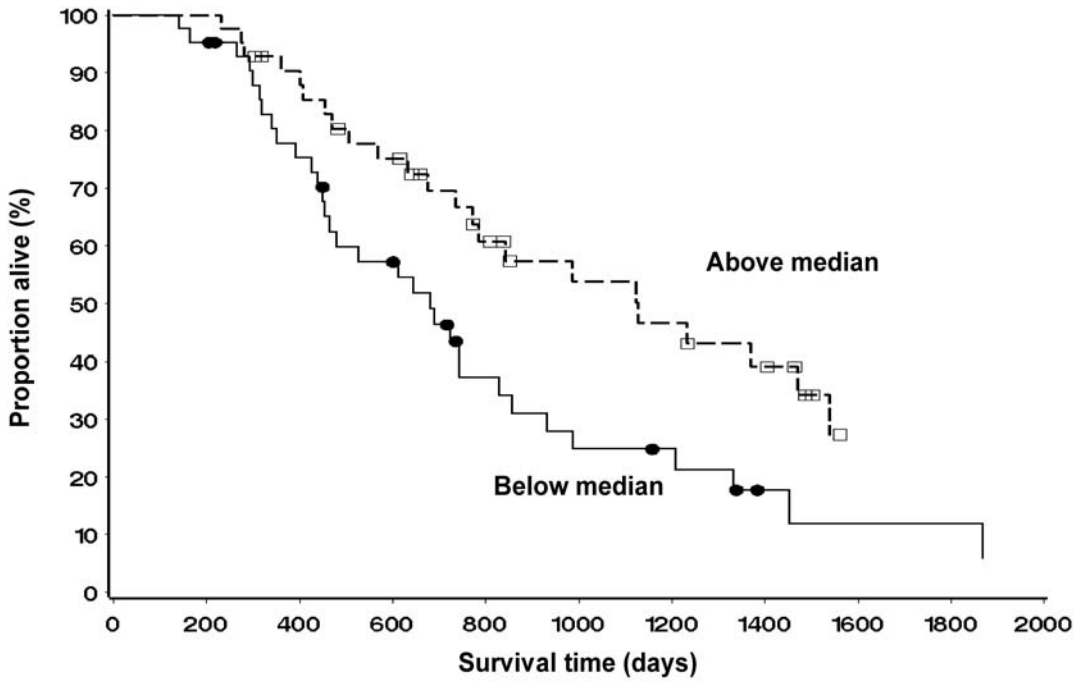

Figure 1. *Disease-specific survival in 86 colorectal cancer (CRC) patients based on the nuclear area (NA) above and below the median value.*

observations on this subject were reported by Ikeguchi *et al.* (31), who found that the 10-year survival of patients with large mean NA in their tumours was lower than that of patients with small nuclei. Clearly, more work based on larger cohorts of patients needs to be carried out to fully elucidate this issue. These inconsistencies may arise, in part, as a consequence of differences in the patients included in the cohorts and/or in the different treatments regimens. Further, different practices in reporting the morphometric data, *i.e.*, the difference between the mean and median values, might be responsible, at least in part, for the reported controversies in the literature. We believe that in this case, using the median values (rather than an arbitrarily selected value) as the cut-off gives an unbiased estimate on the prognostic influence of NA in CRC.

Also of interest was our observation that the tumours with larger NA were associated with the presence of lymph node metastasis, which needs further assessment. We can anticipate that tumours with larger NA might be more aggressive and more likely to be associated with LN involvement at diagnosis, however such a correlation is in contrast with these same cases being associated with longer disease-specific survival (Figure 1). One feasible explanation might be offered by the higher sensitivity of these tumours to chemotherapy, as shown in the present study. Indeed, in the present series, only 6 patients showed a complete response to chemotherapy, *i.e.*, disappearance of all visible lesions (Table II). Notably, all of these 6 patients had tumours with NA above the median values, strongly suggesting that the tumours with a large NA area are more sensitive to the type of chemotherapy regimen used in the present series. This higher sensitivity to treatment could then explain the better survival of these patients.

This distinction does not seem to hold true invariably, however, because some of the non-responders also had a NA above the median, as did all the complete responders. Interestingly, however, our previous study on these patients also demonstrated that the response to treatment could be predicted by the DNA content of the tumour cells, since the irinotecan-based regimen seemed to be more effective in tumours with the largest number of chromosomal changes (35). It seems feasible to anticipate that a larger NA may be closely associated with an increased DNA content in these cells. In fact, the present data offers circumstantial support to this, while demonstrating that the patients with larger NA were more responsive to treatment and, consequently, had a better survival than those with a NA below the median. Interestingly, Yacoub *et al.* measured NA before, during and after the administration of chemotherapy in patients with locally advanced cervical cancer and described a significant reduction in NA in the responders as compared to non-responders (36). Whether this proves to be the case in CRC as well remains to be demonstrated in a future study.

In conclusion, molecular genetic changes, DNA ploidy and expression of different molecular markers have been proposed as indicators of the malignant potential of

tumours (37-39). The present study adds to this literature some data suggesting that other tools are now available to aid the clinician in identifying those patients who might benefit from specific treatment regimens. Such tools include the quantitative morphometric measurement of the NA in the tumour cells. Using the median values of NA as the cut-off seems to effectively discriminate the patients with advanced CRC, who are likely to respond to irinotecan-based chemotherapy regimens and acquire a prognostic advantage, from those who are non-responsive and demonstrate a rapidly progressive disease.

Acknowledgements

This study has been supported by a grant from the Turku University Hospital Clinical Research Foundation. The authors want to extend special thanks to Mr. Heikki Hiekkanen M.Sc., Biostatistician at the Institute of Clinical Medicine, University of Turku, Finland, for drawing the survival curve.

References

1 Gatta G, Faivre J, Capocaccia R and Ponz de Leon M: Survival of colorectal cancer patients in Europe during the period 1978-1989. Eur J Cancer *34(14)*: 2176-2183, 1998.

2 Repetto L, Venturino A, Fratino L, Serraino D, Troisi G, Gianni W and Pietropaolo M: Geriatric oncology: a clinical approach to the older patient with cancer. Eur J Cancer *39(7)*: 870-880, 2003.

3 Wymenga AN, Slaets JP and Sleijfer DT: Treatment of cancer in old age, shortcomings and challenges. Neth J Med *59(5)*: 259-266, 2001.

4 Franceschi S and La Vecchia C: Cancer epidemiology in the elderly. Crit Rev Oncol Hematol *39(3)*: 219-226, 2001.

5 Donohue JH, Williams S, Cha S, Windschitl HE, Witzig TE, Nelson H, Fitzgibbons RJ Jr, Wieand HS and Moertel CG: Perioperative blood transfusions do not affect disease recurrence of patients undergoing curative resection of colorectal carcinoma: a Mayo/North Central Cancer Treatment Group study. J Clin Oncol *13(7)*: 1671-1678, 1995.

6 Greenlee RT, Hill-Harmon MB, Murray T and Thun M: Cancer statistics. CA Cancer J Clin *51(2)*: 144, 2001.

7 Steele G Jr, Ravikumar TS and Benotti PN: Surgical treatments for recurrent colorectal cancer. Cancer *65(3)*: 723-730, 1990.

8 Bruckner HW and Motwani BT: Chemotherapy of advanced cancer of the colon and rectum. Semin Oncol *18(5)*: 443-461, 1991.

9 Iqbal S and Lenz HJ: Determinants of prognosis and response to therapy in colorectal cancer. Curr Oncol Rep *3(2)*: 102-108, 2001.

10 Iqbal S, Stoehlmacher J and Lenz HJ: Tailored chemotherapy for colorectal cancer: a new approach to therapy. Cancer Invest *22(5)*: 762-773, 2004.

11 Seicean R, Funariu G and Seicean A: Molecular prognostic factors in rectal cancer. Rom J Gastroenterol *13(3)*: 223-231, 2004.

12 Ropponen K, Eskelinen M, Kosma VM, Lipponen P, Paakkinen P and Alhava E: Comparison of classic and quantitative prognostic factors in colorectal cancer. Anticancer Res *16(6B)*: 3875-3882, 1996.

13 Johnston PG: Of what value genomics in colorectal cancer? Opportunities and challenges. J Clin Oncol *1;22(9)*: 1538-1539, 2004.

14 Jalava P, Kronqvist P, Smrzova B, Juntti-Patinen L, Kuopio T and Collan Y: Nuclear volume and breast cancer prognosis. Anticancer Res *21(1B)*: 727-732, 2001.

15 Kronqvist P, Kuopio T, Jalava P and Collan Y: Morphometrical malignancy grading is a valuable prognostic factor in invasive ductal breast cancer. Br J Cancer *18; 87(11)*:1275-1280, 2002.

16 Eskelinen M, Lipponen P, Marin S, Haapasalo H, Makinen K, Ahtola H, Puittinen J, Nuutinen P and Alhava E: Prognostic factors in human pancreatic cancer, with special reference to quantitative histology. Scand J Gastroenterol *26(5)*: 483-90, 1991.

17 Nagashima T, Suzuki M and Nakajima N: Cytologic morphometric approach for the prediction of lymph node involvement in papillary thyroid cancer. Anal Quant Cytol Histol *19(1)*: 49-54, 1997.

18 Zhang YH, Kanamaru H, Oyama N, Miwa Y, Suzuki Y, Akino H, Noriki S and Okada K: Prognostic value of nuclear morphometry on needle biopsy from patients with prostate cancer: is volume-weighted mean nuclear volume superior to other morphometric parameters? Urology *55(3)*: 377-781, 2000.

19 Ikeguchi M, Sakatani T, Endo K, Makino M and Kaibara N: Computerized nuclear morphometry is a useful technique for evaluating the high metastatic potential of colorectal adenocarcinoma. Cancer *15:86(10)*: 1944-1951, 1999.

20 Mitmaker B, Begin LR and Gordon PH: Nuclear shape as a prognostic discriminant in colorectal carcinoma. Dis Colon Rectum *34(3)*: 249-259, 1991.

21 Heimann TM, Cohen RD, Szporn A and Gil J: Correlation of nuclear morphometry and DNA ploidy in rectal cancer. Dis Colon Rectum *34(6)*: 449-454, 1991.

22 Puente Dominguez JL: Prognostic value of optic morphometry in colorectal cancer. An R Acad Nac Med *117(3)*: 469-844, 2000.

23 Glimelius B, Ristamaki R, Kjaer M, Pfeiffer P, Skovsgaard T, Tveit KM, Linne T, Frodin JE, Boussard B, Oulid-Aissa D and Pyrhonen S: Irinotecan combined with bolus 5-fluorouracil and folinic acid Nordic schedule as first-line therapy in advanced colorectal cancer. Ann Oncol *13(12)*: 1868-1873, 2002.

24 Miller AB, Hoogstraten B, Staquet M and Winkler A: Reporting results of cancer treatment. Cancer *1;47(1)*: 207-214, 1981.

25 Baak JPA: Manual of Quantitative Pathology in Cancer Diagnosis and Prognosis. Berlin, Springer-Verlag, pp. 531-532, 1991.

26 Hytiroglou P, Harpaz N, Heller DS, Liu ZY, Deligdisch L and Gil J: Differential diagnosis of borderline and invasive serous cystadenocarcinomas of the ovary by computerized interactive morphometric analysis of nuclear features. Cancer *15:69(4)*: 988-992, 1992.

27 Mulder JW, Offerhaus GJ, de Feyter EP, Floyd JJ, Kern SE, Vogelstein B and Hamilton SR: The relationship of quantitative nuclear morphology to molecular genetic alterations in the adenoma-carcinoma sequence of the large bowel. Am J Pathol *141(4)*: 797-804, 1992.

28 Tosi P, Luzi P, Baak JP, Miracco C, Santopietro R, Vindigni C, Mattei FM, Acconcia A and Massai MR: Nuclear morphometry as an important prognostic factor in stage I renal cell carcinoma. Cancer *58(11)*: 2512-2518, 1986.

29 Pienta KJ and Coffey DS: Correlation of nuclear morphometry with progression of breast cancer. Cancer *1;68(9)*: 2012-2016, 1991.

30 Nafe R, Fritsch RS, Soudah B, Hamann A and Choritz H: Histomorphometry in paraffin sections of thyroid tumours. Pathol Res Pract *188(8)*: 1042-1048, 1992.

31 Ikeguchi M, Taniguchi T, Makino M and Kaibara N: Reduced E-cadherin expression and enlargement of cancer nuclei strongly correlate with hematogenic metastasis in colorectal adenocarcinoma. Scand J Gastroenterol *35(8)*: 839-846, 2000.

32 Ambros RA, Pawel BR, Meshcheryakov I, Kotrotsios J, Lambert WC and Trost RC: Nuclear morphometry as a prognostic indicator in colorectal carcinoma resected for cure. Anal Quant Cytol Histol *12(3)*: 172-176, 1990.

33 Hamilton PW, Wyatt JI, Quirke P, Watt PCH, Arthur K, Ward DC and Johnston D: Morphometry of gastric carcinoma: its association with patient survival, tumour stage, and DNA ploidy. J Pathol *168*: 201-208, 1992.

34 Carson HJ, Buschmann RJ, Cintron J and Weisz-Carrington P: Prognostic histomorphometry in colorectal adenocarcinoma. Anal Quant Cytol Histol *17(2)*: 109-112, 1995.

35 Bendardaf R, Lamlum H, Ristamaki R, Algars A, Collan Y and Pyrhonen S: Response to chemotherapy (irinotecan plus 5-fluorouracil) in colorectal carcinoma can be predicted by tumour DNA content. Oncology *66(1)*: 46-52, 2004.

36 Yacoub SF, Shaeffer J, El-Mahdi AM, Faris L and Zhu A: Nuclear morphometry as a predictor of response to neoadjuvant chemotherapy plus radiotherapy in locally advanced cervical cancer. Gynecol Oncol *54(3)*: 327-332, 1994.

37 Iino H, Fukayama M, Maeda Y, Koike M, Mori T, Takahashi T, Kikuchi-Yanoshita R, Miyaki M, Mizuno S and Watanabe S: Molecular genetics for clinical management of colorectal carcinoma. 17p, 18q, and 22q loss of heterozygosity and decreased DCC expression are correlated with the metastatic potential. Cancer *73(5)*: 1324-1331, 1994.

38 Cohn KH, Ornstein DL, Wang F, LaPaix FD, Phipps K, Edelsberg C, Zuna R, Mott LA and Dunn JL: The significance of allelic deletions and aneuploidy in colorectal carcinoma. Results of a 5-year follow-up study. Cancer *79(2)*: 233-244, 1997.

39 Caldes T, Iniesta P, Vega FJ, de Juan C, Lopez JA, Diaz-Rubio E, Fernandez C, Cerdan J, Balibrea JL and Benito M: Comparative survival analysis of *p53* gene mutations and protein accumulation in colorectal cancer. Oncology *55(3)*: 249-257, 1998.

Received February 21, 2005
Accepted May 17, 2005