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Cell Cycle Parameters and Prognosis of Colorectal Cancer*

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Abstract—In vitro *determination of S phase duration and labeling index were performed, in tumor and normal tissues, in 15 patients with rectal and colon cancer to determine if these cell cycle parameters can predict the clinical course of the disease. Microscopic analyses of the tumor and adequate follow-up were obtained for all patients. S phase duration and labeling index did not exhibit any obvious correlations with age, sex, tumor localization, Duke's classification or other microscopic prognostic features; neither did they show any difference between patients alive without cancer 5 yr after initial treatment and those dead from cancer or other causes.*

INTRODUCTION

DETERMINATION of prognostic factors in cancer patients is of paramount importance for planning treatment approaches and long-term evaluation of the disease. In colorectal cancer most prognostic factors, such as penetration through the wall of the intestine or lymph node involvement, are related to the extent of the disease at the time of resection. These features can be objectively defined and the mechanisms by which they influence prognosis are relatively clear. Others, such as the grade of the neoplasm and degree of inflammatory cell reaction, are probably revealing the biologic nature of the tumor and the response of the host. These features are more subjective and the relationship to prognosis is not as well substantiated [1]. Overall, there is a need for more accurate methods for predicting clinical course.

Tumor growth has been related to prognosis [2]. Long tumor doubling times seem to be associated with long survivals in patients with pulmonary metastases [2, 3]. However, the clinical estimation of tumor doubling time requires measurable tumors and a rather long period of observation, and can therefore rarely be done [4].

Cell cycle parameters reflect to a certain extent tumor growth [2]. Using *in vitro* methods, they can be measured easily [4]. Whether they can be related to prognosis appears worth investigating. In 300 solid tumors, including different histologic types collected from the literature, the mean L.I. correlates with the mean doubling time, which in turn correlates with survival [2]. A similar correlation was suggested in breast cancer [5].

This pilot study was undertaken to determine if the measure of labeling index (L.I.: the number of cells labeled by 'pulse-labeling' with [^{3}H]-thymidine) and duration of the phase of DNA synthesis (S phase) is feasible and can be of help in predicting the clinical course in patients with rectal and colon cancer undergoing surgery.

MATERIALS AND METHODS

S phase duration and L.I. were determined in 15 patients with rectal and colon cancer. Adequate follow-up until death, time of recurrence or for at

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least 5 yr since treatment of the primary tumor started could be obtained in all cases. One biopsy sample was taken from each tumor and from adjacent normal mucosa through a rectoscope or in the operating room immediately following resection. All colon cancers were treated by surgery alone, whereas 8 out of 9 rectal cancers were treated preoperatively by radiotherapy, with or without 5-fluorouracil. One patient with rectal cancer, 77 yr old, was treated by radiotherapy alone (No. 11, Table 1). In all these cases biopsies were taken before treatment was initiated. Surgery was curative in all cases.

Details of methods for incubation and autoradiography have been previously reported [6–8]. Each sample was divided into fragments and incubated *in vitro* with 0.5 then after 1 hr with 40 μCi/ml [^{3}H]-thymidine (CEN-Mol Belgium, 28 Ci/mmol). Sections from this labeled material were submitted to autoradiographic and histologic procedures. To confirm that the dose range of [^{3}H]-thymidine was appropriate to this material, the distribution curve of the number of grains per cell obtained with a single dose was established on the cancer in one patient (Fig. 1). In all other cases the labeling intensity was further controlled by scoring 20–30 cells for each dose level. S phase duration and L.I. were then measured. The autoradiographs were examined to establish the proportion of lightly (NL) and heavily labeled nuclei (NH) among the population of labeled cells. S phase duration was calculated from the relationship $NH/NL = S/t$ [9]. The L.I. was measured in the same material by scoring as labeled the heavily labeled cells only.

Anatomopathological examination of the resected colon was performed in all operated cases and extent of the disease determined according to Duke's classification [10]. Histology of each sample was reviewed by one of us (VdH). Degree of cell differentiation, atypia and inflammation were classified in three classes (+, ++, +++), based on general impression of importance of the process. Inflammatory cell reaction was always determined in non-ulcerated areas. The mean number of mitoses per field was established on 10 fields and classified: +, 0–4; ++, 4–10; +++, >10 mitoses per field.

RESULTS

Patient characteristics and cell cycle parameters by survival status are given in Table I. There were ten women and five men with a median age of 61 yr (range 48– 77 yr); eight had a rectal cancer, six a colon cancer and one (No. 3) had both localizations simultaneously. Two patients died from cancer after 3 and 12 months (No. 3 and 12) and two others died from surgical complications after 1.5 and 7 months (Nos 14 and 15). All remaining cases are alive and cancer-free more than 5 yr after initial treatment.

Median S phase duration and L.I. was 18.8 hr (range 7.9–26.8 hr), 32.1% (range 13.7–50.4%) for cancer and 9.8 hr (range 7.3–14.5 hr), 15.6% (range 9.1–33.0%) for adjacent normal mucosa.

Three prognostic categories were established according to five standard microscopic features graded + to +++ (Table 2). Patients were classified in a prognostic category if at least three out of five microscopic features were in agreement. One patient (No. 3) with simultaneous rectal and colon cancer was classified twice, in different prognostic categories. None of the patients had all five criteria concordant, five had four, nine had three and two had two. In these latter two cases Duke's classification determined the prognostic category. With this classification five patients had a bad prognosis, of which one died from cancer (No. 13) and one died from surgical complications (No. 15). Six had intermediate prognosis, of which one died from cancer (No. 12). Four had a good prognosis, of which one died from surgical complications (No. 14). The mean S phase duration and L.I. for tumors with bad, intermediate and good prognosis were respectively 19.2 hr, 36.2%; 19.0 hr, 30.8%; and 18.2 hr, 26% (no significant differences). Mean S phase duration and L.I. of normal mucosa near tumors with bad, intermediate and good prognosis were respectively 12.3 hr, 12.3%; 9.1 hr, 13.1%; and 10 hr, 33% (no significant differences). Cell cycle parameters by patient characteristics are given in Table 3. S phase duration and L.I. did not vary significantly with age, sex, tumor localization or Duke's classification.

Cell cycle parameters by survival status are given in Table 4. No difference in this respect emerges between patients dead with or without cancer and patients alive, nor between patients dead with and without cancer, but the number of deaths is obviously too small to draw any conclusion at this stage.

DISCUSSION

Tumor growth is the resultant effect of proliferation and cell loss. A global measure of the growth rate as estimated by tumor doubling time might predict the clinical course of cancer. However, this kind of calculation is rather cumbersome and has only been applied in selected situations [1, 11, 12]. Although some correlations have been established between doubling time, L.I. and cell loss [2], it is generally admitted that potential doubling time established on the basis of measured cell cycle parameters without knowledge of cell loss has no clinical value [13].

Table 1. Patient characteristics and cell cycle parameters by survival status

Survival status	No.	Sex	Age	Site of primary	Anatomopathological data: Duke's	Differentiation	Cellular atypia	Cellular inflammatory reaction	Mitosis	Cell cycle parameters: S (hr) Tumor	S (hr) Normal mucosa	L.I. (%) Tumor	L.I. (%) Normal mucosa
	1	F	60	colon	A	+++	+	+	++	26.6	10	24.7	33.0
	2	F	48	rectum	A	++	+++	+	+	-	-	29.5	-
	3	M	61	rectum	A	+++	++	++	++	17.0	9.5	24.1	25.0
				colon	A	+	+	+	+++	18.2	10.2	32.1	12.3
Alive	4	F	66	colon	B	+++	++	++	++	21.6	9.6	31.3	24.6
without	5	F	61	rectum	B	++	+++	+	+++	-	-	35.0	-
cancer	6	F	54	Colon	B	++	++	++	+++	23.5	8.5	24.5	12.2
	7	M	72	rectum	B	++	+++	+++	+	17.0	7.3	39.7	15.7
	8	F	64	rectum	B	-	-	-	-	18.8	9.0	32.8	10.9
	9	M	61	colon	B	++	+	+	++	21.5	11.3	23.4	12.2
	10	M	65	colon	C	++	++	+	+++	22.0	14.5	50.4	15.6
	11*	F	77	rectum	-	+++	+	+	+	20.0	-	30.9	-
Dead with cancer	12	M	61	colon	B	++	++	+	+++	13.7	8.8	39.5	28.6
	13	F	54	rectum	C	+	+++	+	+	-	-	13.7	9.1
Dead without	14	F	68	rectum	A	+	++	+	+	7.9	-	18.9	-
cancer	15	F	69	rectum	B	+	+++	+	+	17.5	-	49.8	-
									Mean	18.9	9.9	31.3	18.1
									(S.D.)	(4.7)	(1.9)	(10.1)	(8.2)
										$P < 0.01$		$P < 0.01$	

*Patient treated by radiotherapy alone.

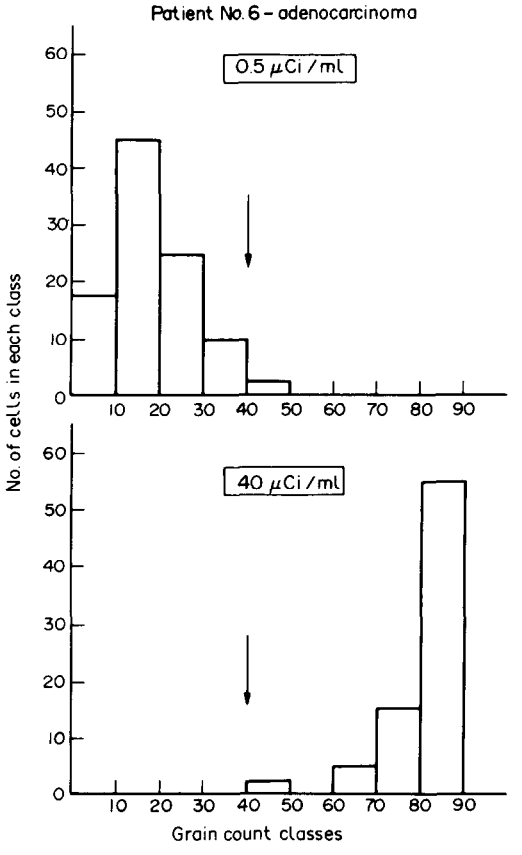

Fig. 1. Frequency histograms of grain count over labeled nuclei in autoradiographs of adenocarcinoma incubated for 15 min *with two different doses of* [^{3}H]*-thymidine. Arrow indicates, in double labeled material, the upper limit chosen for the weakly labeled cell population. This produced* 4% *misplaced weakly labeled cells and, in this case, no misplaced heavily labeled cells.*

Whether cell proliferation or cell loss by themselves are accurate prognostic factors remains unanswered. If proliferation is implicitly correlated to growth, cell loss might be the most important factor determining an increase in tumor size. In some tumor types up to 90% of the produced cells are destroyed [14]. Cell loss probably varies largely from one tumor type to another and within the same tumor type, depending on local growth conditions and host defense mechanisms. Its extent, at least on an individual basis, cannot be determined.

On the contrary, cell proliferation parameters using autoradiographic or flow cytometric analyses are easily determined [6, 7, 15] and thus remain the only available method based on tissue analysis to estimate tumor growth and, potentially, to predict the clinical course. Some attempts were made to test this possibility. In colon cancer L.I. was found to increase with Duke's stage in a series of 20 patients [15]. In breast cancer L.I. did not correlate with the number of axillary metastases but with the interval between primary therapy and relapse, and with the duration of survival after relapse. Low L.I.s were associated with good prognostic factors, i.e. older age, minimal nuclear anaplasia and estrogen receptor positivity [5]. Cell proliferation is usually estimated by a measure of the L.I. This holds true only if S phase duration remains unchanged. In this work, using an *in vitro* double labeling technique with two different levels of [^{3}H]-thymidine, S phase duration and L.I. were simultaneously determined in 15 patients with colorectal cancer. As previously reported [6], S phase durations are longer and L.I.s are higher than in adjacent normal mucosa (Table 1). These differences are statistically significant ($P < 0.01$ by paired t tests).

An increased S phase duration appears to be a marker of cancer [1]. As only 1 of 15 tumors (No. 14) had a low S phase duration, one may, when confronted with premalignant tissue, consider a >12-hr S phase duration as suspicious

Table 2. Cell cycle parameters and prognosis according to microscopic prognostic criteria

Prognosis (prognostic criteria)	No. of concordant criteria for each patient (pt.) 5	4	3	2	Mean S phase (hr)/mean L.I. (%) Tumor	Normal mucosa
Bad [Duke's C, C Dif +, CA +++, CIR +, Mit +++]*	-	pt 13†	pt 3 (C)‡ pt 15 pt 5 pt 10		19.2/36.2	12.3/12.3
Intermediate [Duke's B, C Dif ++, CA ++, CIR ++, Mit ++]	-	pt 6 pt 4	pt 3 (R)§ pt 9 pt 12†	pt 7 pt 8	19.0/30.8	9.1/18.4
Good [Duke's A, C Dif +++, CA +, CIR +++, Mit +]	-	pt 11 pt 1	pt 2 pt 14		18.2/26	10.0/33.0

*C Dif = cellular differentiation; CA = cellular atypia; CIR = cellular inflammatory reaction; Mit = mitosis.
†Patients dead from cancer.
‡(C) = colon.
§(R) = rectum.

of cancer. In our sample this leaves only one false positive result in the histologically normal mucosas (No. 10).

Tumor L.I. does not seem to be proportional to S phase duration (Table 1). Although it cannot be completely excluded that some increased L.I.s were partially related to an increased S phase duration, this suggests that these tumors have different rates of proliferation either by a difference in growth fraction, a shortening of cell cycle or a combination of both.

Cell cycle parameters were not correlated to sex, age, tumor localization or the usual prognostic factors (Tables 1 and 3). L.I. seemed to correlate with prognostic classification based on histopathological data (mean L.I.s are 36.2, 30.8 and 26.0% respectively for bad, intermediate and good prognosis), but these findings are not statistically significant and larger series are needed to certify them. Moreover, there are notable exceptions: for example, the patient cumulating the worse prognostic factors and who died within 3 months after curative surgery had the lowest L.I. (No. 13, Table 1).

Our results do not reproduce results showing a trend of the L.I. to increase with Duke's stage [15]. It may be asked whether L.I. and tumor extension should necessarily be correlated. In fact, extension of the disease at surgery depends more on factors like tumor localization, duration of symptoms or host defense mechanisms than on proliferation. Moreover, one could suggest an opposite hypothesis. Admitting that tumor tissue remains, to some extent, sensitive to regulatory mechanisms, an increased L.I. might reflect partial destruction of the tumor through host defense mechanisms and a compensatory increase of proliferation. The compensatory process could be compared to what was observed in inflammatory bowel disease [16] in normal mucosa in contact with carcinogens [17], or in tumors partially destroyed by radiotherapy or chemotherapy [18, 19]. This would explain the fact that in two of our patients classified as bad prognosis, No. 13, with a low L.I. of 13.7%, relapsed and died shortly after initial treatment, while No. 10, with a high L.I. of 50.4%, was alive more than 5 yr after surgery. Similar findings were reported by others, who showed a 26.5% L.I. in a patient with localized tumor compared to 10.9% when lymph nodes and blood vessels were invaded [20]. However, it must be kept in mind, when interpreting these results, that *in vitro* estimation of cell cycle parameters carried out on small tissue samples after *in vitro* or *in vivo* procedures are not necessarily representative of the whole tumor.

There is no doubt that a more accurate prediction of clinical course in cancer patients

Table 3. Cell cycle parameters by patients' characteristics

Cell cycle parameters	Age (No.)*		Sex (No.)		Tumor localization (No.)		Duke's classification (No.)		
	<60 yr	>60 yr	Men	Women	Rectum	Colon	A	B	C
Mean duration of S phase (hr)	25.0(2)	17.7(11)	18.2(6)	19.4(7)	16.4(6)	21.0(7)	17.4(4)	19.1(7)	22.0(1)
Mean percentage of labeled cells	23.5(4)	33.9(12)	34.8(6)	29.1(10)	30.4(9)	32.2(7)	25.8(5)	36.2(8)	32.0(2)

*(No.) = number of measures.

Table 4. Cell cycle parameters by survival status

Cell cycle parameters	Survival status		
	Dead with and without C*/Alive†	Dead with C/dead without C	Dead with C/alive
Mean duration of S phase (hr)	13.1/20.4	13.7/12.9	13.7/20.4
Mean percentage of labeled cells	30.4/31.6	26.6/34.3	26.6/31.6

*C = cancer.
†All living patients are cancer-free.

would be of great help. Attempts were made to demonstrate that L.I. increases with Duke's staging [15]. Our work suggests that, generally, this does not hold true and that, in any case, the measure will not be useful at an individual level. S phase duration does not seem to have any prognostic significance but is, indeed, a marker of cancer [1].

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REFERENCES

1. BUCKWALTER JA, KENT TH. Prognosis and surgical pathology of carcinoma of the colon. *Surg Gynecol Obstet* 1973, **136**, 465–472.
2. TUBIANA M, MALAISE E. Comparison of cell proliferation kinetics in human and experimental tumors: response to irradiation. *Cancer Treat Rep* 1976, **60**, 1887–1895.
3. MORTON DL, JOSEPH WL, KETCHAM AS, GEELHOED GW, ADKINS PC. Surgical resection and adjunctive immunotherapy for selected patients with multiple pulmonary metastases. *Ann Surg* 1973, **178**, 360–366.
4. CHARBIT A, MALAISE EP, TUBIANA M. Relations between the pathological nature and the growth of human tumors. *Eur J Cancer* 1971, **7**, 307–315.
5. MEYER JS, LEE JY. Relationship of S-phase fraction of breast carcinoma in relapse to duration of remission, estrogen receptor content, therapeutic responsiveness, and duration of survival. *Cancer Res* 1980, **40**, 1890–1896.
6. BLEIBERG H, GALAND P. *In vitro* autoradiographic determination of cell kinetic parameters in adenocarcinomas and adjacent healthy mucosa of the human colon and rectum. *Cancer Res* 1976, **36**, 325–328.
7. BLEIBERG H, SALHADIN A, GALAND P. Cell cycle parameters in human colon. Comparison between primary and recurrent adenocarcinomas, benign polyps and adjacent unaffected mucosa. *Cancer* 1977, **39**, 1190–1194.
8. GALAND P, MAINGUET P, ARGUELO M, CHRETIEN J, DOUXFILS N. *In vitro* autoradiographic studies of cell proliferation in the gastrointestinal tract of man. *J Nucl Med* 1968, **2**, 37–39.
9. WEGENER K, HOLLWEG S, MAURER W. Autoradiographische Bestimmung der DNS-Verdopplungszeit und anderer Teilphasen de Zell-Zyklus bei Fetale Zellarten des Ratte. *Z Zellforsch* 1964, **63**, 309–326.
10. MORSON BC, DAWSON IMP. Malignant epithelial tumours. In: MORSON BC, DAWSON IMP, eds. *Gastrointestinal Pathology*. Oxford, Blackwell Scientific Publications, 1979, 539–573.
11. SPRATT JS, SPRATT TL. Doubling time of pulmonary metastases and host survival. *Ann Surg* 1964, **159**, 161–171.
12. WELIN S, YOURER J, SPRATT JS *et al.* The rates and patterns of growth of tumours and recurrence observed serially by double contrast enema study. *Am J Roentgenol* 1963, **90**, 673–687.
13. STEEL GG. The utility of cell kinetic data in the design of therapeutic schedules. *J Radiat Oncol Biol Phys* 1979, **5**, 145–146.
14. FRINDEL E, MALAISE E, TUBIANA M. Cell proliferation kinetics in five human solid tumors. *Cancer* 1968, **22**, 611–620.
15. TEMPLE WJ, SUGARBAKER EV, THORNTHWAITE JT, HENSLEY GT, KETCHAM AS. Correlation of cell cycle analysis with Duke's staging in colon cancer patient. *J Surg Res* 1980, **28**, 314–318.
16. BLEIBERG H, MAINGUET P, GALAND P, CHRETIEN J, DUPONT-MAIRESSE N. Cell renewal in the human rectum. "*In vitro*" autoradiographic study on active ulcerative colitis. *Gastroenterology* 1970, **58**, 851–855.
17. OEHLERT W. Cellular proliferation in carcinogenesis. *Cell Tissue Kinet* 1973, **6**, 325–335.
18. FRINDEL E, CROISAT H, VASSORT F. Stimulating factors liberated by treated bone marrow. *In vitro* effect on CFU Kinetic. *Exp Hematol* 1976, **6**, 315–318.
19. TUBIANA M. On kinetics of cell proliferation and cancer. *Bull Cancer* 1978, **65**, 407–416.
20. LIEB LM, LISCO H. *In vitro* uptake of tritiated thymidine by carcinoma of the human colon. *Cancer Res* 1966, **26**, 733–740.