Flow Cytometric DNA Ploidy and S-Phase Fraction Correlate with Histopathologic Indicators of Tumor Behavior in Colorectal Carcinoma

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BACKGROUND: The clinical behavior of colorectal carcinoma is highly variable without reliable predictive biomarkers. Previous reports have shown that flow cytometric DNA analysis may provide valuable prognostic information in these tumors. PURPOSE AND METHODS: This study evaluates the DNA ploidy and the S-phase fraction (SPF) on frozen samples obtained from 61 patients with colorectal carcinoma by using flow cytometry, and it correlates the data with histopathologic features known to affect disease prognosis. Tumors were classified according to the World Health Organization’s histologic criteria and were staged according to the American Joint Committee on Cancer’s classification system. Grade of the neoplasm, vascular invasion, and perineural tumor spread were evaluated in every case. RESULTS: Fifty-nine percent of tumors were aneuploid and showed statistically significant higher S-phase values than diploid tumors (22.5 vs. 11.2 percent; P < 0.00001). Mean SPF of the whole series was 17.9 (range, 4.2-44.2) percent. A statistically significant association was found between SPF values and histologic grade (P < 0.0016), nodal status (P < 0.0007), distant metastasis (P < 0.0001), tumor stage (P < 0.0001), venous invasion (P < 0.0002), and lymphatic permeation (P < 0.01) but not with perineural growth and infiltration of the neoplasm through the bowel wall (T). DNA ploidy correlated positively with tumor stage (P < 0.03), and the association between aneuploidy and advanced stages of the disease was statistically significant. CONCLUSIONS: These findings showed that flow cytometric DNA ploidy and SPF, evaluated in fresh samples, are potentially useful parameters to estimate colorectal carcinoma biopathology. Aneuploidy and high replicative neoplastic activity correlated with histopathologic features that are commonly associated with the prognosis of colorectal carcinoma, being SPF-related to disease dissemination and, therefore, an indicator of clinical relevance. [Key words: DNA ploidy; S-phase fraction; Flow cytometry; Colorectal carcinoma; Prognosis]


THE clinical behavior of colorectal carcinoma is highly variable without predictable markers of disease outcome. Histopathologic staging still remains the most valuable prognostic factor of the neoplasm.1-5 However, it does not accurately identify patients at risk of recurrence and fatal evolution, especially those bearing intermediate stages of the tumor. Among new prognostic indicators that might help to select subgroups of patients who could potentially benefit from adjuvant therapy, interest has been devoted to DNA ploidy and cell cycle S-phase fraction.

Flow cytometric analysis has been extensively used in colorectal carcinoma to evaluate tumor cell nuclear ploidy. Aneuploid tumors are commonly associated with a worse prognosis,6-14 but several studies have shown lack of correlation between a DNA ploidy pattern and clinical outcome.15-21 Conflicting results can be explained among others by the lack of standardization of technical procedures and the extent of DNA intratumoral heterogeneity.22

Some authors have suggested that cell kinetics can be a valuable biologic parameter in anticipating the progression of several human tumor types.23 Cell proliferation, as estimated by the S-phase fraction (SPF), has been less extensively studied than DNA ploidy in colorectal carcinoma.

The present study was designed to evaluate the S-phase fraction as a marker of tumor cell proliferative activity and the nuclear DNA ploidy in a series of 61 colorectal carcinomas using flow cytometric analysis of fresh material. Additionally, the results were corre-
lated with histopathologic parameters commonly associated with tumor prognosis.

MATERIALS AND METHODS

Patients and Clinical Data

Frozen samples of surgical specimens from 61 patients with colorectal carcinoma who underwent surgery at the Instituto Português Oncologia-Lisbon Centre between 1989 and 1993 were included in this study. Clinical data were obtained from medical records. Mean age of patients was 59.5 (range, 35–84) years, and 34 were males and 27 were females. None of the patients received preoperative radiotherapy. Localization of tumors was as follows: rectum (36–59 percent), left colon (13–21.3 percent), and right colon (12–19.7 percent).

Histopathologic Data

Formalin-fixed, paraffin-embedded tissue was used for histopathologic analysis. All cases were classified on hematoxylin and eosin-stained slides using the World Health Organization’s classification24 and were staged according the American Joint Committee on Cancer’s criteria.25

Neoplasms were graded as follows: well differentiated (23–37.7 percent), moderately differentiated (32–52.5 percent), and poorly differentiated (6–9.8 percent). Ten patients had no invasion beyond the tunica muscular propria: 5 tumors (8.2 percent) were circumscribed to the submucosa (T1) and 5 (8.2 percent) showed invasion of the muscular propria limited to the external layer (T2). In 51 patients, there was invasion below the muscular propria: in 45 (73.8 percent) the tumor involved the subserosa or the non-peritonealized pericolic or perirectal tissues (T3) and in 6 patients (9.8 percent) the tumor infiltrated adjacent structures (T4). The average number of lymph nodes evaluated per case was 7.3. Forty tumors had no regional lymph nodes metastasis (N0, 65.6 percent), and 21 cases had positive nodes: 14 had metastasis in one to three pericolic or perirectal lymph nodes (N1, 22.9 percent) and 7 had four or more pericolic and/or perirectal positive nodes (N2, 11.5 percent). Seven neoplasia had distant metastasis (M1, 11.5 percent). Venous invasion, lymphatic permeation, and perineural spread were assessed in all cases. Thirty-seven tumors (60.7 percent) showed permeation of the lymphatic vessels, 5 (8.2 percent) had venous invasion, and there was perineural tumor spread in 11 (18.0 percent).

DNA Flow Cytometry Study

For flow cytometric study, fragments of the primary tumor were immediately frozen in liquid nitrogen and stored at −80°C. All samples were assessed for the presence of nonneoplastic colorectal tissue by microscopic evaluation of frozen sections. For DNA analysis,26 frozen samples containing at least 20 percent tumor cell representativity were used, subsequently thawed, and mechanically sliced into small pieces in cold phosphate-buffered saline (PBS) using scalpels. The homogenate was left for two to three minutes in a glass tube to allow sedimentation of tissue fragments, and the turbid supernatant was then recovered. The cellular suspension was washed twice in PBS by centrifugation at 150 × g for ten minutes, and cells were counted with a hemocytometer. For DNA staining, cellular suspensions were incubated with propidium iodide (50 μg/ml) in Tris-MgCl₂ buffer for one hour in the dark at room temperature and treated with RNase (1 mg/ml) in PBS and 0.05 percent non-ionic detergent NP40. Immediately before flow cytometric analysis, specimens were injected through a 27-gauge needle and then filtered through a 55 μm nylon mesh to avoid cell aggregates. The stained nuclei were analyzed on an Epics Profile II flow cytometer (Coulter Electronics, Hialeah, FL) equipped with a 488 nm argon-ion laser. DNA-specific fluorescence emission was measured using a 575 nm band pass filter. The flow cytometer was considered calibrated when coefficients of variation less than 2 percent were obtained with fluorescent microspheres (DNA Check lot 5948, Coulter Corp.). Chicken red blood cells were used as an internal control and helped with identification of the G0/G1 diploid population.27 A minimum of 20,000 nuclei were collected per run without any gating condition and recorded on a single-parameter 256-channel integrated fluorescence histogram.

Cell cycle analysis of DNA histograms was performed using the Multicycle software (Phoenix Flow Systems, San Diego, CA), developed by PS Rabino-vitch (University of Washington, Seattle, WA), based on the mathematical method of Dean and Jett.28 A correction for cellular debris and clumps was made using a “sliced nuclei” curve fitted to the background to the left of the G0/G1 peak and to the right of the G2 M peak. Tumor proliferative activity was expressed in