Abnormalities in the Expression of Cell Cycle-related Proteins in Tumors of the Small Bowel[1]

Nadir Arber,[2] Hanina Hibshoosh, Wataru Yasui, Alfred I. Neugut, Aharon Hibshoosh, Yao Yao, Alessandro Sgambato, Hirofumi Yamamoto, Itzhak Shapira, David Rosenman, Ina Fabian, I. Bernard Weinstein, Eiichi Tahara, and Peter R. Holt

Gastrointestinal Oncology Unit, Department of Gastroenterology, Tel Aviv Sourasky Medical Center [N. A., D. R.] and Department of Histology and Cell Biology, Tel-Aviv University [N. A., A. S., I. S., D. R., I. F.], Tel Aviv 64239, Israel; the Herbert Irving Comprehensive Cancer Center [H. H., A. I. N., Y. Y., I. B. W., P. R. H.], Departments of Pathology [H. H., B. W.] and Medicine [A. I. N., I. B. W., P. R. H.], and School of Public Health [A. I. N.], College of Physicians and Surgeons, Columbia University and Division of Gastroenterology, St. Luke's-Roosevelt Hospital Center [P. R. H.], New York, New York 10032; Division of Biostatistics, San Jose University California [A. H.]; and First Department of Pathology, Hiroshima University School of Medicine, Hiroshima 565-0875, Japan [W. Y., H. Y., E. T.]

Abstract

Tumors of the small bowel are quite rare for unknown reasons, although they resemble colorectal tumors in many respects. The purpose of this study was to determine whether abnormalities in the expression of several cell cycle control genes are of importance in small bowel tumorigenesis by comparing a series of samples of normal mucosa, adenomatous polyps, and adenocarcinomas. The levels of cyclin D1, cyclin E, p16, p21, p27, and p53 proteins were determined by immunohistochemistry in samples of normal small bowel ($n = 16$), small bowel adenomas ($n = 20$), and small bowel adenocarcinomas ($n = 24$). Normal small bowel mucosa expressed p27 protein, but not the other cell cycle-related proteins. About 20% of the tumors displayed a decrease in the expression of this protein. The most frequent alteration in the tumors was an increase in the p16 protein. Increased expression of p53 was associated with tumor progression because it was overexpressed in 45% of the adenomas and 65% of the adenocarcinomas ($P < 0.05$). Advanced age and increased detection of cyclin D1 and p53 were associated with a decreased 3-year survival ($P < 0.05$). Cell cycle abnormalities are early and important events in the multistep process of small bowel tumorigenesis, thus resembling colorectal carcinogenesis. As in colon cancer, deregulated expression of G_1 proteins may perturb cell cycle control in benign adenomas of the small bowel and thereby enhance tumor progression. Increased expression of cell cycle inhibitors in tumors may serve as a defense mechanism for tumor progression.

Introduction

Neoplasms of the small intestine account for only 1–5% of GI[3] tumors, despite the fact that the small intestine makes up 75% of the total GI mucosal surface area. Thus, the small intestine is remarkably resistant to the development of benign or malignant neoplasms, particularly when compared to the high incidence rates of adenocarcinoma of the colon. The reasons for the relatively rare occurrence of small bowel adenocarcinomas are not known. It has been suggested that although the small bowel may be exposed to carcinogens similar to those responsible for colorectal cancer, it may have protective mechanisms against tumor formation (1–20).

Worldwide, the incidence rates of small intestinal adenocarcinomas parallel those of colorectal adenocarcinomas. Colorectal and small bowel adenocarcinoma also share other features as well (6). The incidence of both types of carcinoma appears to be elevated in individuals with Peutz-Jeghers syndrome (7, 8), celiac disease (6, 8–10), cystic fibrosis (9), Crohn's disease (7, 11, 12), HNPCC syndrome (13), and familial adenomatous polyposis (7, 8, 16). A morphologic adenoma-carcinoma sequence has been described in the small intestine (7), similar to the well-described sequence in the colon and rectum (21–26). Both types of cancer also share certain epidemiological features, such as an increased incidence in the Western hemisphere (4, 15, 20), a possible association with cholecystectomy (7), and a possible association with high fat and protein intake (9).

Uncontrolled cell proliferation is the hallmark of cancer, and there is increasing evidence that tumor cells have acquired damage to genes that directly regulate their cell cycle (27–31). Genetic alterations affecting the G_1 phase of the cell cycle are so frequent in human cancers that abnormalities in this pathway may actually be necessary for tumor development. Like the tumor suppressor protein p53, other components of the G_1 phase may participate in checkpoint functions that regulate homeostatic tissue renewal throughout life. Oncogenic processes often exert their effects by targeting specific regulators of the G_1-phase progression (28, 29). During the G_1 phase, cells respond to extracellular signals by either advancing toward S phase and cell division, withdrawing from the cycle into a

Received 3/31/99; revised 9/9/99; accepted 9/14/99.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by funding from the Israel Cancer Association (to N. A.), the Dorot Foundation, and the National Dairy Council (to N. A. and P. R. H.), an award for the Cancer Research from FUSO Pharmaceutical Co. Osaka, Japan; (to H. Y.), the United States National Cancer Institute (Grant RO1-CA63467), and awards from the National Foundation for Cancer Research and the Lucille Markey Charitable Trust (to I. B. W.). This work was performed in partial fulfillment of PhD thesis requirement of N. A. (Tel-Aviv University, Sackler Faculty of Medicine).
[2] To whom requests for reprints should be addressed, at Director, Gastrointestinal Oncology Unit, Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel 64239. Phone: 972-3-6974968/280; Fax: 972-3-6974622; E-mail: narber@post.tau.ac.il, nadir@tasmc.health.co.il.

[3] The abbreviations used are: GI, gastrointestinal; HNPCC, hereditary nonpolyposis colorectal cancer.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/12/1101/3256536/ce12990110 1p.pdf by guest on 01 March 2024

Table 1 Cell cycle abnormalities in small bowel tumors

	Cyclin D1	Cyclin E	p16	p21	p27	p53
Adenomas	31%[a]	31%	92%	50%	83%	47%
Adenocarcinomas	30%	38%	91%	46%	77%	65%

[a] The percentage of patients with detectable levels of the protein.

resting state, or undergoing apoptosis (30, 31). Unlike transit through the S, G_2, and M phases, G_1 progression normally relies on stimulation by mitogens and can be blocked by antiproliferative cytokines.

Cancer cells tend to lose these normal control mechanisms and remain in the cycle. Because cell cycle exit can facilitate maturation and terminal differentiation, these processes are also subverted as well. However, these aspects have not been examined in detail in tumors of the small bowel. In previous studies, our group (32–34) and other investigators (35) found that the cell cycle control gene, *cyclin D1*, plays an important role in the multistep process of colorectal carcinogenesis. Recently, Rashid and Hamilton (36) reported that point mutations in the c-K-*ras* proto-oncogene and alterations in the *p53* gene were common genetic events in small bowel carcinomas, similar to large bowel carcinomas. However, in contrast to colorectal cancer, loss of *APC* and *DCC* were rare events in small bowel cancers. Alteration of the transforming growth factor β RII gene was not present even in HNPCC tumors. However, our group did observe similar molecular genetic abnormalities in large bowel and small bowel tumors (18–20, 37).

The aim of the present study was to determine whether abnormalities in the expression of several cell cycle control genes are of importance in small bowel tumorigenesis by comparing a series of samples of normal mucosa, adenomatous polyps, and adenocarcinomas obtained from the small bowel. The proteins examined were p16, p21, p27, p53, cyclin D1, and cyclin E.

Materials and Methods

Case Material. Cases of small bowel adenomatous polyps and adenocarcinomas were identified by searching the surgical pathology computer files of two major hospitals in New York City, the Columbia-Presbyterian Medical Center and St. Luke's-Roosevelt Hospital Medical Center. Tissue from the tumors had been removed endoscopically or obtained at surgery during the period between January 1, 1985 and December 31, 1996 and processed by routine clinical histopathological methods, including fixation with formalin and paraffin embedding. We did not include tissues from familial adenomatous polyposis or HNPCC patients or metastatic lesions from other sites or from Whipple pancreatoduodenectomy specimens in this study. Follow-up information was obtained from medical record review from the hospital tumor registries and from yearly contacts with the treating physicians or patients. The 3-year survival was obtained from the hospital records or by telephone contact with the patient's physician or family.

The study population is summarized in Table 1. The 16 normal intestinal samples were normal biopsy specimens obtained from the duodenums of patients who were endoscoped for other reasons, and their histologies were normal. A total of 20 small bowel adenomas and 24 small bowel adenocarcinoma samples were examined, but not all markers were examined in all of these cases.

Immunohistochemistry. All immunohistochemical analyses were performed with an avidin-biotin complex immunoperoxidase technique. Five-μm tissue sections were mounted on poly-L-lysine-coated slides. After deparaffinization in Americlear (Baxter, McGaw Park, IL) and absolute ethanol, sections were hydrated through a series of graded alcohol, distilled water, and PBS at pH 7.4. Slides were then immersed in 10 mM citrate buffer (pH 6) and microwaved (to enhance antigen exposure) for a total of 10 min at 750 W. After blocking the tissue with goat or horse serum for 20 min, primary antihuman antibodies were added and incubated overnight at 4°C in a high-humidity chamber. These included rabbit polyclonal antibodies to cyclin D (Upstate Biotechnology, Lake Placid, NY) and $p16^{ink4}$ (PharMingen, San Diego, CA) and mouse monoclonal antibodies to $p21^{waf1}$ (Santa Cruz Biotechnology, Santa Cruz, CA), $p27^{Kip1}$ (Transduction Laboratories, Lexington, KY), and p53 (Immunotech, Inc., Westbrook, ME). Although all the concentrations of primary antibodies gave good nuclear staining, the optimal concentration that gave a minimal background was 5 mg/ml for cyclin D1 and p53 and 2 mg/ml for cyclin E, p16, p21, and p27. Positive controls were breast adenocarcinoma for cyclin D1 and p53 and gastric adenocarcinoma for cyclin E, p16, p21, and p27.

Subsequent steps used the Vectastain rabbit Elite ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Color development was accomplished with a 0.375 mg/dl solution of a 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) containing 0.003% hydrogen peroxide. Slides were counterstained with hematoxylin and dehydrated, and coverslips were applied using Acrytol mounting medium (Surgipath Medical Industries, Richmond, IL).

The specificity of the antibodies was demonstrated (data not shown) by inhibition of immunohistochemical staining in positive controls by preincubating the antibody with 1 mg of the immunizing peptides for 1 h at 4°C, representing about a 100-fold excess of peptide over antibody.

Interpretation of Immunohistochemical Staining. Staining for cyclin D1 and p53 was interpreted by one author (H. H.), and staining for cyclin E, p16, p21, and p27 was interpreted by another author (W. Y.). Both of these individuals are experienced surgical pathologists. Nuclear staining was considered positive if the chromogen was clearly detected in at least 10% of the nuclei within a microscopic field. Up to 5% of the nuclei at the base of the crypt were positive. However, this represents less than 0.1% of the nuclei in an average microscopic field. Positive and negative controls were included within each batch of slides. To confirm reproducibility, 25% of the slides were randomly chosen and scored twice in the same batch, and all batches were coded and blindly scored at least twice. Duplicate slides gave similar results (data not shown).

Statistical Analysis. A set of 58 samples from 44 patients was collected. Taking a conservative approach in our analysis, only a single sample from each patient was randomly selected for the analysis. The proportions of samples expressing the different genes from different histological categories were computed and then compared across categories for selected factors (including gender, age, cigarette smoking, alcohol consumption, location of the lesion within the small bowel, differentiation, Duke's stage, and the presence of dysplasia). When comparing proportions positive for staining across histological categories, the Fisher and χ^2 tests were used (38). McNemar's test (39) was also conducted for testing asymmetry in the association between the different genetic markers.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/12/1101/3256536/ce12990110 1p.pdf by guest on 01 March 2024

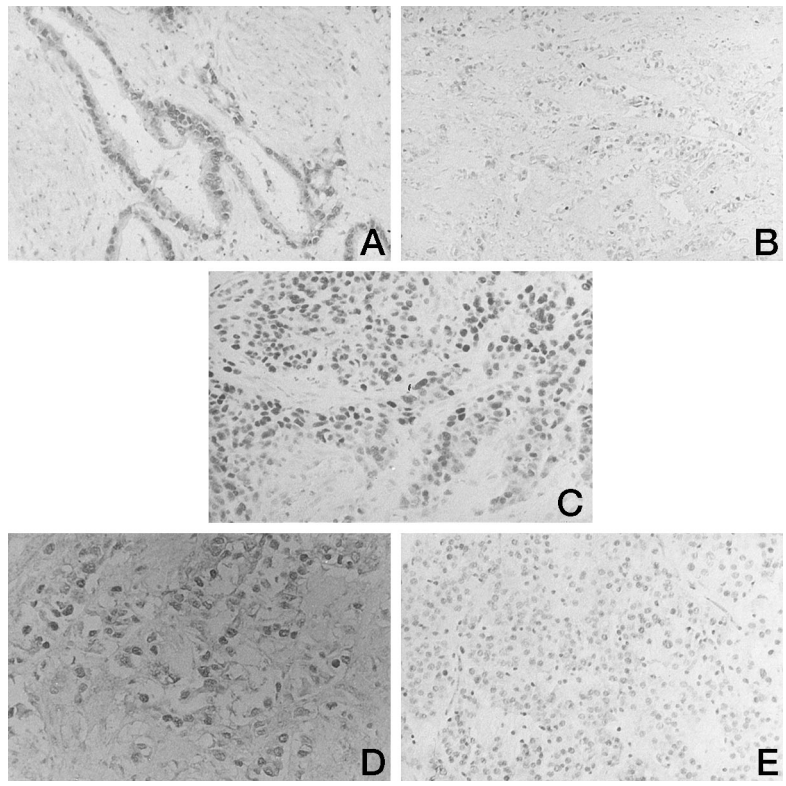

Fig. 1. Positive nuclear staining in small bowel adenocarcinoma: *A*, p16; *B*, p21; *C*, p27; *D*, cyclin D1; and *E*, cyclin E.

Results

The demographic and clinical characteristics of our cases are consistent with those of small bowel adenocarcinoma patients described previously in the literature (1–20). Thus, these tumors were predominantly found in males in the sixth or seventh decade of life, and in 80% of patients, they were confined to the duodenum. The patients ranged in age from 28–92 years. Sixteen of the patients studied were female, and 28 were male.

All of the normal-appearing mucosa samples expressed the $p27^{kip1}$ protein. None of these samples displayed appreciable nuclear staining for $p16^{ink4a}$, p21, p53, cyclin D1, or cyclin E. Rarely, in about 1 cell per 20 crypts, positive nuclear staining for these proteins was noted in cells near the base of the crypt.

The abnormalities identified in the small intestinal tumors are summarized in Table 1. The most common alteration involved an increase in the $p16^{ink4a}$ protein, which was overexpressed in 92% of the adenomas and 91% of the adenocarcinomas. Increased p53 protein was observed in 47% of the adenomas and in 65% of the adenocarcinomas ($P < 0.05$), suggesting a significant increase with tumor progression. There was also increased expression of the cyclin D1, cyclin E, p21, and $p27^{kip1}$ proteins in both the adenomas and adenocarcinomas, but there was no evidence that these markers increased with tumor progression (Table 1). The $p27^{kip1}$ protein, which was seen in all of the normal mucosa samples, was not detected in 17% of the adenomas and 23% of the adenocarcinomas (Fig. 1).

Possible associations with demographic data and lifestyle characteristics are shown in Table 2. Increased expression of cyclin D1 in small bowel neoplasms was associated with advanced age ($P < 0.05$). Increased p53 levels were more prevalent in tumors from females (71% *versus* 37%, $P < 0.05$). Caucasians showed a trend toward increased expression of cyclin E (39%) and p53 (65%) when compared to non-Caucasians (17% and 43%, respectively). All of the tumors in nonsmokers showed increased expression of p16, p27 was undetectable in only 14% of the cases. Cigarette smoking showed a statistical trend toward an association with cyclin D1 overexpression. Moreover, only 64% of the smokers demonstrated increased levels of p16, and down-regulation of p27 was found in as many as 43% of smokers. Alcohol consumption did not show an association with any of the cell cycle abnormalities.

Tumor stage and grade were not significantly associated with any of the above abnormalities. Advanced age was associated with poorer survival ($P < 0.05$), but race, gender, and alcohol and tobacco consumption were not. Increased expression of cyclin D1 and p53 proteins was associated with a decrease in the 3-year survival. Of those subjects who died within the 3-year period, 7 of 15 (47%) showed increased expression of cyclin D1, and 11 of 14 had mutant p53 (74%). The levels of cyclin E and the inhibitors p16, p21, and p27 did not predict outcome.

Increased expression of cyclin D1 was closely associated with mutated p53 in the adenomas, and increased expression of cyclin E protein was closely associated with increased p53 in adenocarcinomas ($P < 0.05$). We did not find any significant correlation between the levels of p53 and p21 or between other markers. The lack of correlation between p53 and p21 may reflect the fact that several factors, in addition to p53, can affect the level of p21.

Although cyclin D1 is generally considered to be a nuclear protein, four adenocarcinomas and one adenoma (i.e., 11% of the tumor samples) displayed strong supranuclear cytoplasmic immunostaining, which was not seen in the other tumors or in the normal-appearing mucosa samples. The specificity of the cytoplasmic staining was confirmed in three ways: (*a*) addi-

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/12/1101/3256536/ce12990110 1p.pdf by guest on 01 March 2024

Table 2 Association with demographic and lifestyle factors

	Cyclin D1[a]	Cyclin E	p53	p16	p21	p27
Sex						
Male	5/16 (31%)	2/10 (20%)	6/16 (38%)	6/8 (75%)	4/8 (50%)	6/8 (75%)
Female	9/28 (32%)	8/21 (38%)	17/24 (71%)	17/17 (100%)	10/19 (53%)	15/18 (83%)
Race						
Non-White	5/18 (29%)	2/12 (17%)	6/14 (43%)	8/9 (89%)	5/9 (56%)	9/11 (82%)
White	9/27 (33%)	8/19 (42%)	17/26 (65%)	15/16 (94%)	9/18 (50%)	12/15 (80%)
Age						
<Median[b]	5/21 (24%)	7/17 (41%)	9/19 (47%)	14/15 (93%)	9/15 (60%)	11/14 (79%)
>Median	9/23 (39%)	3/14 (21%)	14/21 (67%)	9/10 (90%)	5/12 (42%)	10/12 (83%)
Alcohol						
No	7/21 (33%)	5/15 (33%)	12/20 (60%)	12/13 (92%)	8/15 (53%)	11/14 (79%)
Yes	3/9 (33%)	3/7 (43%)	6/9 (67%)	6/6 (100%)	2/5 (40%)	4/5 (80%)
Smoking						
Negative	5/20 (25%)	5/16 (27%)	10/18 (56%)	13/13 (100%)	8/15 (53%)	12/14 (86%)
Positive	6/15 (40%)	3/10 (30%)	8/15 (53%)	7/11 (64%)	4/7 (57%)	4/7 (57%)

[a] Positive cases/total number of cases (%).
[b] Median age, 65 years.

tional sections from the same block confirmed the observation; (*b*) inhibition of the cytoplasmic staining in positive cases was achieved by preincubating the antibody with 1 mg of the immunizing peptides; and (*c*) the cytoplasmic staining was seen by two independent observers.

Discussion

This is the first detailed description of abnormalities in the expression of several cell cycle control proteins in small bowel tumors.

As in colorectal carcinogenesis (21–27, 32, 40), increased expression of cyclin D1 and p27 and down-regulation of p16 and p21 are all early events in the multistep process of small bowel carcinogenesis because they were seen at similar frequencies in both small bowel adenomas and adenocarcinomas. In addition, increased levels of the p53 protein, which presumably reflect inactivating mutations, are a late event in small intestine tumorigenesis.

The demonstration of cyclin D1 expression (and not that of the other cell cycle proteins) in the cytoplasm of some of the small bowel tumors is of particular interest. This cytoplasmic staining for cyclin D1 was reproducible. There were no distinguishing characteristics in these samples including medical and demographic data or the expression of the different proteins. There were no statistically significant characteristics of this group. This might be due to the small size of the group ($n = 5$). Cytoplasmic staining for cyclin D1 has also been seen previously in other types of cancers. It was seen in a subset of human tumors of the esophagus (41), stomach (41), colon (32), breast,[4] and lymphomas (42). This is unlikely to represent leakage of this protein from the nucleus, because it occurred in three of five small bowel tumors in the absence of nuclear staining. The presence of cyclin D1 outside the nucleus suggests that cyclin D1 may have an additional, presently undefined physiological role. It will be of interest to determine whether any of the small bowel tumors, which displayed increased expression of cyclins D1 and/or E or decreased expression of the cell cycle inhibitors, have amplified copies or deletions of the corresponding genes.

Abnormalities in the above genes may play an important role in patient prognosis. Thus, increased expression of cyclin D1 in pancreatic adenocarcinoma (43) and decreased expression of p27 in gastric (44) and colonic adenocarcinomas (45) were associated with a poorer prognosis. Cyclin D1 amplification has also been associated with a poor prognosis in esophageal squamous cell carcinomas (40). Cyclin E overexpression correlates with the stage of gastric (41) and colorectal carcinoma (46). Decreased expression of p21 correlates with the invasiveness and aggressiveness of gastric cancer (47, 48). In the present study, increased expression of cyclin D1 or p53 in patients with small bowel adenocarcinomas was associated with a significantly decreased 3-year survival.

The possible significance of a negative association during the multistep process between increased levels of p16 and p21 with two possible risk factors for small bowel tumors, namely, cigarette smoking and alcohol consumption, remains to be determined.

In another study, submitted elsewhere, the percentage of ras mutations in the entire GI tract was evaluated. Ras mutations were not associated with the expression of cyclin D1, cyclin E, p16, p21, or p27.

In summary, it is of interest that small bowel tumors share not only epidemiological features with large bowel tumors but also several abnormalities in the expression of cell cycle-related proteins and, indeed, with other tumors of the GI tract as well. These findings are, in general, consistent with previous comparable studies on genetic abnormalities in small and large bowel tumors (18–20, 36, 37). However, the reasons for the relative rarity of small bowel tumors remain to be determined.

[4] N. Arber and H. Hibshoosh, unpublished observations.

References

1. Ross, R. K., Hartnett, N. M., Bernstein, L., and Henderson, B. E. Epidemiology of adenocarcinomas of the small intestine: is bile a small bowel carcinogen? Br. J. Cancer, *63:* 143–145, 1991.

2. Ashley, S. W., and Wells, S. A. Tumors of the small intestine. Semin. Oncol., *15:* 116–128, 1998.

3. Kim, S. H., Roth, K. A., Moser, A. R., and Gordon, J. I. Transgenic mouse models that explore the multistep hypothesis of intestinal neoplasia. J. Cell Biol., *123:* 877–893, 1993.

4. Lowenfels, A. B. Why are small bowel tumors so rare? Lancet, *1:* 24–26, 1973.

5. DiSario, J., Burt, R. W., Vargas, H., and McWhorter, W. Small bowel cancer: epidemiological and clinical characteristics from a population-based registry. Am. J. Gastroenterol., *89:* 699–701, 1994.

6. Neugut, A. I., and Santos, J. The association between cancers of the small and large bowel. Cancer Epidemiol. Biomark. Prev., *2:* 551–553, 1993.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/12/1101/3256536/ce12990110 1p.pdf by guest on 01 March 2024

7. Sellner, F. Investigation on the significance of the adenoma-carcinoma sequence in the small bowel. Cancer (Phila.), *66:* 702–715, 1990.

8. Johnson, A. M., Harman, P. K., and Hanks, J. B. Primary small bowel malignancies. Am. Surg., *51:* 31–36, 1985.

9. Lowenfels, A. B., and Sonni, A. Distribution of small bowel tumors. Cancer Lett., *3:* 83–86, 1997.

10. Nielsen, S. N. J., and Wold, L. E. Adenocarcinoma of jejunum in association with nontropical sprue. Arch. Pathol. Lab. Med., *110:* 822–824, 1986.

11. Chen, C. C., Neugut, A. I., and Rotterdam, H. Risk factors for adenocarcinomas and malignant carcinoids of the small intestine: preliminary findings. Cancer Epidemiol. Biomark. Prev., *3:* 205–207, 1993.

12. Lashner, B. A. Risk factors for small bowel cancer in Crohn's disease. Dig. Dis. Sci., *37:* 1179–1184, 1992.

13. Vasen, H. L. A., Mecklin, J.-P., Merra Khan, P., and Lynch, H. T. The international collaborative group on hereditary non-polyposis colorectal cancer (ICG-HNPCC). Dis. Colon Rectum, *34:* 424–425, 1991.

14. Bhutani, M. S., and Gopalswamy, N. A multicenter experience in the United States with primary malignant tumors of the small intestine. Am. J. Gastroenterol., *89:* 460, 1994.

15. Chow, J. S., Chen, C. C., Ahsan, H., and Neugut, A. I. A population-based study of the incidence of malignant small bowel tumors: SEER, 1973–1990. Int. J. Epidemiol., *25:* 722–728, 1996.

16. Seifert, E., Schulte, F., and Stolte, M. Adenoma and carcinoma of the duodenum and papilla of Vater: a clinicopathologic study. Am. J. Gastroenterol., *87:* 37–42, 1992.

17. Perzin, K. H., and Bridge, M. F. Adenomas of the small intestine: a clinicopathologic review of 51 cases and a study of their relationship to carcinoma. Cancer (Phila.), *48:* 799–819, 1981.

18. Arber, N., Neugut, A. I., Weinstein, I. B., and Holt, P. R. Molecular genetics of small bowel cancer. Cancer Epidemiol. Biomark. Prev., *6:* 745–748, 1997.

19. Arber, N. Small bowel adenocarcinoma. Oncology (Basel), *11:* 549, 1997.

20. Neugut, A. I., Jacobson, J. S., Suh, S., Mukherjee, R., and Arber, N. The epidemiology of cancer of the small intestine. Cancer Epidemiol. Biomark. Prev., *6:* 745–748, 1997.

21. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Presinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Bos, J. L. Genetic alterations during colorectal tumor development. N. Engl. J. Med., *319:* 525–532, 1988.

22. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.

23. Hamilton, S. R. The molecular genetics of colorectal neoplasia. Gastroenterology, *105:* 3–7, 1993.

24. Ilyas, M., and Thomlinson, I. P. M. Genetic pathways in colorectal cancer. Histopathology, *28:* 389–399, 1996.

25. Reale, M. A., and Fearon, E. R. Gene defects in colorectal tumorigenesis. *In:* G. P. Young, P. Rozen, and B. Levin (eds.), Prevention and Early Detection of Colorectal Cancer, pp. 63–86. London: W. B. Saunders Company, Ltd., 1996.

26. Kinzler, K. W., and Vogelstein, B. Lessons from hereditary colorectal cancer. Cell, *87:* 159–170, 1996.

27. Pines, J. Cyclins: wheels within wheels. Cell Growth Differ., *2:* 305–310, 1991.

28. Hunter, T., and Pines, J. Cyclins and cancer. II. Cyclin D and CDK inhibitors come of age. Cell, *79:* 573–582, 1994.

29. Hall, M., and Peters, G. Genetics alterations of cyclins, cyclin-dependent kinases, and CDK inhibitors in human cancer. Adv. Cancer Res., *68:* 67–108, 1996.

30. Pardee, A. B. GI events and regulation of cell proliferation. Science (Washington DC), *246:* 603–608, 1989.

31. Sherr, C. J. G_1 phase progression: cycling on cue. Cell, *79:* 551–555, 1994.

32. Arber, N., Hibshoosh, H., Moss, S. F., Sutter, S., Zhang, Y., Begg, M., Wang, S., Weinstein, I. B., and Holt, P. R. Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. Gastroenterology, *110:* 669–674, 1996.

33. Arber, N., Doki, Y., Han, E. K. H., Zhou, P., Sgambato, A., Kim, N. W., Holt, P. R., and Weinstein, I. B. Antisense to cyclin D1 inhibits the growth and reduces the tumorigenicity of human colon cancer cells. Cancer Res., *57:* 1569–1574, 1997.

34. Sutter, T., Doi, S., Carnevale, K. A., Arber, N., and Weinstein, I. B. Expressions of cyclins D1 and E in human colon adenocarcinomas. J. Med., *28:* 285–309, 1997.

35. Bartkova, J., Lukas, J., Strauss, M., and Bartek, J. The prad-1\cyclin D1 oncogene product accumulates aberrantly in a subset of colorectal carcinomas. Int. J. Cancer, *58:* 568–573, 1994.

36. Rashid, A., and Hamilton, S. R. Genetic alterations in sporadic and Crohn's-associated adenocarcinomas of the small intestine. Gastroenterology, *113:* 127–135, 1997.

37. Sutter, T., Arber, N., Moss, S. F., Findling, R. I., Neugut, A. I., Weinstein, I. B., and Holt, P. R. Frequent c-K-*ras* mutations in small bowel adenocarcinomas. Dig. Dis. Sci., *41:* 115–116, 1996.

38. Brier, S. S. Analysis of contingency tables under cluster sampling. Biometrika, *67:* 591–596, 1980.

39. McNemar, Q. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika, *12:* 153–157, 1994.

40. Jiang, W., Kahn, S. M., Tomita, N., Zhang, Y. J., Lu, S. H., and Weinstein, I. B. Amplification and expression of the human cyclin D1 gene in esophageal cancer. Cancer Res., *52:* 2980–2983, 1993.

41. Arber, N., Gammon, M. D., Hibshoosh, H., Britton, J. A., Zhang, Y., Schoenberg, J. B., Rotterdam, H. N., Fabian, I., Holt, P. R., and Weinstein, I. B. Overexpression of cyclin D1 occurs in both squamous carcinomas and adenocarcinomas of the esophagus and in adenocarcinomas of the stomach. Hum. Pathol., *30:* 1087–1092, 1999.

42. Banno, S., Yoshikawa, K., Nakamura, S., Yamamoto, K., Seito, T., Nitta, M., Takahashi, T., Ueda, R., and Seto, M. Monoclonal antibody against PRAD1/cyclin D1 stains nuclei of tumor cells with translocation or amplification at the BCL-1 locus. Jpn. J. Cancer Res., *85:* 918–926, 1994.

43. Gansauge, S., Ramadani, M., Stobbe, H., Rau, B., Harada, N., and Beger, H. G. Overexpression of cyclin D1 in human pancreatic carcinoma is associated with poor prognosis. Cancer Res., *57:* 1634–1637, 1997.

44. Yasui, Y., Kudo, Y., Semba, S., Yokozaki, H., and and Tahara, E. Reduced expression of cyclin-dependent kinase inhibitor $p27^{kip1}$ is associated with advanced stage and invasiveness of gastric carcinomas. Jpn. J. Cancer Res., *88:* 625–629, 1997.

45. Loda, M., Cukor, B., Tam, S. W., Lavin, P., Fiorentino, M., Draetta, G. F., Jessup, J. M., and Pagano, M. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nat. Med., *3:* 231–234, 1997.

46. Yasui, W., Kuniyasu, H., Yokozaki, H., Semba, S., Shimamoto, F., and Tahara, E. Expression of *cyclin* E in colorectal adenomas and adenocarcinomas: correlation with Ki-67 antigen expression and abnormal accumulation of p53. Virchows Archiv., *429:* 13–19, 1996.

47. Tahara, E., Yasui, W., Yokozaki, H., and Shimamoto, F. Molecular diagnosis of gastrointestinal cancers: the application to clinical practice. Int. J. Clin. Oncol., *1:* 63–68, 1996.

48. Yasui, W., Akama, Y., Kuniyasu, H., Semba, S., Shimamoto, F., and Tahara, E. Expression of cyclin-dependent kinase inhibitor p21 in non-neoplastic mucosa and neoplasia of the stomach: relation with p53 status and proliferative activity. J. Pathol., *180:* 122–128, 1996.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/12/1101/3256536/ce1299011101p.pdf by guest on 01 March 2024