# Nonsteroidal Anti-inflammatory Drug Use Associated with Reduced Incidence of Adenocarcinomas of the Esophagus and Gastric Cardia that Overexpress Cyclin D1: A Population-Based Study

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## Abstract

**This study was undertaken to determine whether selected risk factors for esophageal and gastric cancer are associated with tumors that overexpress cyclin D1. Archived tumor tissue was available for 630 esophageal and gastric cancer patients who participated in a population-based case-control study. Patients were categorized into case groups based on whether protein overexpression of the cyclin D1 gene, as assessed by immunohistochemistry, was present (cyclin D1+, $n = 285$) or not (cyclin D1−, $n = 345$) in the tumor. The distribution of risk factors in each of these case groups was then compared with the distribution among the 695 controls. Multivariate-adjusted odds ratios (OR) for esophageal adenocarcinoma were reduced in relation to use of aspirin and other nonsteroidal anti-inflammatory drug (NSAID) use but only among patients with cyclin D1+ tumors (0.45, 95% confidence interval [CI] = 0.26, 0.79) and not among those with cyclin D1− tumors (1.12, 95% CI = 0.67, 1.86). A similar pattern was observed for gastric cardia adenocarcinomas. In contrast, ORs for esophageal squamous cell carcinoma and noncardia gastric adenocarcinomas in relation to NSAID use were reduced, regardless of cyclin D1 status. ORs did not vary with cyclin D1 status in relation to alcohol, body size, or cigarette smoking, with the following exception; for noncardia gastric adenocarcinomas the cyclin D1− tumors showed a 2-fold elevation in the OR with ever smoking. These data suggest that the reduction in risk associated with NSAID use may be restricted to those esophageal and gastric cardia adenocarcinomas that overexpress cyclin D1.**

## Introduction

The cell cycle control gene cyclin D1 plays a critical role in regulating the $G_1$ to S transition of the cell cycle, thus influencing cellular proliferation, differentiation, and carcinogenesis. Cyclin D1 gene overexpression has been detected in a variety of human cancers and can function as a cellular oncogene (1). The prevalence of cyclin D1 protein overexpression, determined by immunohistochemical methods, has been reported (2) to be high in esophageal squamous cell carcinoma (71%), esophageal adenocarcinoma (64%), gastric cardia adenocarcinoma (50%), and noncardia gastric adenocarcinomas (50%). Prevalence (46%) is also high in Barrett's esophagus (3), suggesting that increased expression of cyclin D1 is an early-stage event in the development of esophageal adenocarcinoma and that the increased expression of this gene may predispose the epithelium to malignant transformation (2, 3). Recent prospective studies have confirmed that biopsy specimens that stain positive for cyclin D1 overexpression from patients with Barrett's esophagus, as compared with those that did not, were more likely to develop esophageal adenocarcinoma (4, 5).

We previously reported (6) a decrease in the odds ratio (OR) for esophageal cancer in relation to the use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs), an observation that has been confirmed in a recent meta-analysis (7). The underlying mechanisms of these potential chemopreventives have not been clearly delineated; however, several are likely including cyclooxygenases 1- (COX1) and 2- (COX2) dependent as well as COX1- and COX2-independent actions. A recent laboratory study (8) reported that, in a mouse model of oral-esophageal cancers that express cyclin D1, administration of sulindac in the drinking water reduced the occurrence of epithelial tumors by 50%.

The molecular epidemiology study reported here was undertaken to explore whether the overexpression of cyclin D1 in tumors of patients with esophageal and gastric cancer was more strongly associated with NSAID use, or other risk factors for

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these cancers, than among patients with tumors that did not overexpress cyclin D1.

## Materials and Methods

This study draws on data collected as part of a collaborative multicenter, population-based case-control study that was conducted in the following three geographic locations: the state of Connecticut; a fifteen-county area of New Jersey state; and a three-county area of western Washington state. The primary aim of the parent project was to identify risk factors for esophageal adenocarcinoma and gastric cardia adenocarcinoma and to compare these factors with those known to increase the risk of esophageal squamous cell carcinoma and other gastric adenocarcinomas. The methods of the parent study have been described in more detail previously (9). This investigation was performed with approval from our institutional review boards and in accord with an assurance filed with and approved by the United States Department of Health and Human Services.

**Study Population.** Eligible case subjects were men and women between 30 and 79 years of age who were diagnosed with primary invasive cancer of the esophagus or stomach from February 1, 1993 through January 31, 1995 in Connecticut; April 1, 1993 through November 30, 1994 in New Jersey; and from March 1, 1993 through February 28, 1995 in Washington. All case subjects diagnosed with adenocarcinomas of the esophagus or gastric cardia ("target case subjects") were considered eligible for the study. Persons diagnosed with squamous cell carcinoma of the esophagus or adenocarcinomas located elsewhere in the stomach ("comparison case subjects") were sampled by frequency matching to the expected distribution of the target case subjects on geographic area and 5-year age group in Connecticut, New Jersey, and Washington; on sex in New Jersey and Washington; and on race in New Jersey.

Population-based control subjects were frequency matched to the expected distribution of target case subjects by 5-year age group and sex. Control subjects who were 30–64 years of age were identified using a modification of Waksberg's random digit dialing method (10); those who were 65–79 years of age were identified by random sampling of rosters from the Health Care Financing Administration (now called Centers for Medicare and Medicaid Services).

Interviews were obtained for 554 (81%) of the eligible target case subjects, 589 (74%) of the eligible comparison case subjects, and 695 (74%) of the eligible control subjects. The overall response rate among control subjects was 70% when the telephone screener response rate of 91% is taken into account for the 52% of control subjects that were identified by use of random digit dialing. The primary reason for nonparticipation was subject refusal (12% of target case subjects, 17% of comparison case subjects, and 23% of control subjects) followed by physician refusal among case subjects (4% for each group). Interviews were completed with the study subject, rather than to the closest next of kin (usually the spouse), for 70% of the target case subjects, 68% of the comparison case subjects, and 97% of the control subjects.

**Risk Factor Information.** During the in-person interview, a structured questionnaire was administered by a trained interviewer, and the average time to complete the questionnaire was 130 min. Information was collected on demographic characteristics, tobacco and alcohol use, other beverage consumption, medical history, use of medications, diet, and occupational history. Definitions of the variables used in these analyses have been described previously in more detail (6, 7, 11–13).

*Table 1* Distribution of selected subject characteristics by availability of slides for tumor staining among cases in a population-based case-control study of esophageal and gastric cancer in Connecticut, New Jersey, and Washington, 1993–1995

| Characteristic | Cases without slides available $N = 513$ (44.9%) | Cases with slides available $N = 630$ (55.1%) | $\chi$-square $P$-value |
|---|---|---|---|
| Geographic center | | | 0.001 |
| Connecticut | 208 (57.5%) | 154 (42.5%) | |
| New Jersey | 170 (32.6%) | 352 (67.4%) | |
| Washington | 135 (52.1%) | 124 (47.9%) | |
| Age (in years) | | | 0.237 |
| <50 | 59 (48.8%) | 62 (51.2%) | |
| 50–59 | 86 (46.5%) | 99 (53.5%) | |
| 60–69 | 172 (47.4%) | 191 (52.6%) | |
| 70–79 | 196 (41.4%) | 278 (58.7%) | |
| Sex | | | 0.465 |
| Male | 398 (44.3%) | 500 (55.7%) | |
| Female | 115 (46.9%) | 130 (53.1%) | |
| Race | | | 0.836 |
| Black | 43 (47.8%) | 47 (52.2%) | |
| Others | 17 (46.0%) | 20 (54.0%) | |
| White | 453 (44.6%) | 563 (55.4%) | |
| Cigarette smoking | | | 0.603 |
| Never | 114 (46.3%) | 132 (53.7%) | |
| Ever | 399 (44.5%) | 498 (55.5%) | |
| Alcohol intake | | | 0.371 |
| Never | 123 (47.3%) | 137 (52.7%) | |
| Ever | 390 (44.2%) | 493 (55.8%) | |
| Body mass index (in quartiles)[a] | | | 0.468 |
| <23.01 | 131 (46.1%) | 153 (53.9%) | |
| 23.01–25.06 | 122 (48.0%) | 132 (52.0%) | |
| 25.06–27.29 | 117 (41.8%) | 163 (58.2%) | |
| 27.29+ | 139 (43.4%) | 181 (56.6%) | |
| NSAID[b] use | | | 0.638 |
| Never | 325 (43.9%) | 415 (56.1%) | |
| Ever | 168 (45.4%) | 202 (54.6%) | |

[a] Quartiles based on distribution in controls (males and females combined).
[b] NSAID, nonsteroidal anti-inflammatory drug.

**Retrieval of Archived Tumor Tissue.** For the present study, archived tumor blocks with sufficient tumor for the assays were retrieved for 630 (55.1%) of the 1130 cancer cases. Availability of the usable archived tumor tissue varied little by tumor location and/or histology or stage at diagnosis (data not shown). Cases with sufficient tumor tissue available for the laboratory assays did not systematically differ from those without such tissue with regard to known and suspected risk factors for these tumor types (Table 1).

**Laboratory Assays.** Cyclin D1 overexpression was determined by immunohistochemical techniques using methods described previously (3, 14). Briefly, 5-$\mu$m sections from formalin-fixed, paraffin-embedded tissue sections were deparaffinized, hydrated, and placed in 10 mM citrate buffer (pH 6) and microwaved for a total to 10 min (antigen retrieval). Blocking serum (horse serum), primary antibody (mouse monoclonal IgG2a), and the antihuman cyclin D1 (1:20; Immunotech) were used. The detection method was the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). The chromogen diaminobenzidine was used and sections counterstained with methyl green.

The study pathologist (H. Hibshoosh) was responsible for the interpretation of the immunohistochemical results using the same methodology as in studies reported previously (3, 15). Nuclear staining of cyclin D1 was evaluated in the esophageal and gastric carcinomas by a semiquantitative scoring system

*Table 2* Adjusted[a] odds ratio (OR) and 95% confidence intervals (CI) for cyclin D1 positive (+) and cyclin D1 negative (−) esophageal and gastric cancer in relation to cigarette smoking by tumor type among men and women in Connecticut, New Jersey, and Washington, 1993–1995

| Tumor type | Controls ($N$ = 695) | Cyclin D1+ cases | | Cyclin D1− cases | | Ratio of the ORs (95% CI) |
|---|---|---|---|---|---|---|
| | | $N$ = 285 | OR (95% CI) | $N$ = 345 | OR (95% CI) | |
| All esophageal and gastric cancers | | | | | | |
| Never | 244 | 60 | 1.0 | 72 | 1.0 | |
| Ever | 451 | 225 | 1.95 (1.39, 2.74) | 273 | 2.21 (1.60, 3.07) | 0.88 (0.59, 1.31) |
| Current | 155 | 93 | 2.39 (1.60, 3.58) | 103 | 2.63 (1.78, 3.87) | 0.91 (0.58, 1.44) |
| Former | 296 | 132 | 1.72 (1.19, 2.48) | 170 | 2.01 (1.42, 2.84) | 0.86 (0.56, 1.31) |
| Esophageal adenocarcinoma | | | | | | |
| Never | 244 | 15 | 1.0 | 20 | 1.0 | |
| Ever | 451 | 65 | 2.63 (1.45, 4.77) | 59 | 1.81 (1.02, 3.22) | 1.45 (0.66, 3.18) |
| Current | 155 | 22 | 2.67 (1.31, 5.43) | 21 | 1.92 (0.96, 3.86) | 1.39 (0.54, 3.54) |
| Former | 296 | 43 | 2.61 (1.40, 4.88) | 38 | 1.75 (0.95, 3.24) | 1.49 (0.65, 3.40) |
| Gastric cardia adenocarcinoma | | | | | | |
| Never | 244 | 12 | 1.0 | 18 | 1.0 | |
| Ever | 451 | 43 | 1.73 (0.90, 3.35) | 76 | 2.15 (1.24, 3.74) | 0.80 (0.35, 1.83) |
| Current | 155 | 18 | 2.06 (0.96, 4.46) | 27 | 2.40 (1.25, 4.61) | 0.85 (0.33, 2.23) |
| Former | 296 | 25 | 1.56 (0.77, 3.17) | 49 | 2.03 (1.13, 3.64) | 0.77 (0.32, 1.85) |
| Esophageal squamous cell carcinoma | | | | | | |
| Never | 244 | 9 | 1.0 | 3 | 1.0 | |
| Ever | 451 | 82 | 3.79 (1.84, 7.82) | 23 | 3.96 (1.03, 15.16) | 0.95 (0.22, 4.14) |
| Current | 155 | 47 | 7.19 (3.78, 15.74) | 12 | 6.23 (1.45, 26.69) | 1.16 (0.24, 5.78) |
| Former | 296 | 35 | 2.34 (1.08, 5.09) | 11 | 2.98 (0.73, 12.07) | 0.79 (0.16, 3.64) |
| Noncardia gastric adenocarcinomas | | | | | | |
| Never | 244 | 24 | 1.0 | 31 | 1.0 | |
| Ever | 451 | 35 | 0.90 (0.51, 1.61) | 115 | 2.37 (1.50, 3.74) | 0.38 (0.19, 0.75) |
| Current | 155 | 6 | 0.53 (0.21, 1.36) | 43 | 2.80 (1.61, 4.87) | 0.19 (0.07, 0.53) |
| Former | 296 | 29 | 1.24 (0.66, 2.31) | 72 | 2.06 (1.25, 3.41) | 0.60 (0.29, 1.25) |

[a] Adjusted for center, race, sex, use of any alcohol (beer, wine, or hard liquor), body mass index, and nonsteroidal anti-inflammatory drug use.

that considers both staining intensity and percentage of positive nuclei. The system assesses nuclear staining intensity as a 4-level ordered categorical variable (0 = none, 1 = mild, 2 = moderate, 3 = strong); the percentage of positive cells is a dichotomous variable where negative = none or rare cells (where rare is defined as ≤5% are positive) and positive = more than rare (*e.g.*, generally >5% of the cells were positive). Cases were scored as cyclin D1 positive (cyclin D1+) only if: (*a*) the staining intensity was either moderate (2) or strong (3) and (*b*) the number of nuclei showing a positive staining exceeded rare cells (generally >5%). This cutoff value for rare cells reflects the level of background staining seen in adjacent normal mucosa. Cases not meeting these criteria were scored as cyclin D1 negative (cyclin D1−).

**Statistical Methods.** Each of the four case tumor groups (esophageal adenocarcinoma, gastric cardia adenocarcinoma, esophageal squamous cell carcinoma, noncardia gastric adenocarcinomas) was compared with the controls. For each tumor group, unordered polytomous unconditional logistic regression (16) was performed to simultaneously calculate the OR (and corresponding 95% confidence intervals [CI]) for cyclin D1+ cases (as compared with the controls) and cyclin D1− cases (as compared with the controls) in relation to potential risk factors for esophageal and gastric cancer. All models included as covariates the frequency-matched factors of geographic center (Connecticut/Washington/New Jersey, entered as indicator variables) and age (in quartiles and entered as indicator variables). Final models included variables for center, age, race, sex, cigarette smoking, use of beer, use of wine, use of hard liquor, body mass index, and use of any NSAID. The ratio of the ORs (comparing the OR for cyclin D1+ cancer to the OR for cyclin D1− cancer), and the corresponding CI was used as an indicator of heterogeneity (17).

## Results

The prevalence of cyclin D1 overexpression as determined by immunohistochemical techniques in this population-based sample of esophageal and gastric cancer cases was 45.2% (285 of 630). There was variation in the prevalence by histological type and/or tumor location, with overexpression evident in 50.3% (80 of 150) of esophageal adenocarcinomas, 36.9% (55 of 149) of gastric cardia adenocarcinomas, 77.8% (91 of 117) of esophageal squamous cell carcinomas, and 28.8% (59 of 205) of noncardia gastric adenocarcinomas. Within tumor subtype, the prevalence of cyclin D1 did not consistently vary with geographic residence, age, or sex; data by race were too sparse among African Americans or other non-whites to yield reliable data (not shown).

The incidence of both cyclin D1+ tumors and cyclin D1− tumors was associated with cigarette smoking in these data (Table 2). There were some differences in the magnitude of the OR according to tumor subtype. For esophageal adenocarcinoma, the OR in relation to ever smoking, as compared with never smoking, was more pronounced for tumors that overexpressed cyclin D1 (OR = 2.63; 95% CI = 1.45, 4.77) than for tumors that did not (OR = 1.81; 95% CI = 1.02, 3.22). However, as reflected in the wide CI, the heterogeneity between the two ORs was not substantial (ratio of the ORs for cyclin D1+/cyclin D1− tumors = 1.45; 95% CI = 0.66, 3.18). For esophageal squamous cell carcinoma, there was little or no difference between the OR for cyclin D1+ tumors and cyclin D1− tumors. For gastric cardia adenocarcinoma, however, the magnitude of the OR in relation to ever smoking was less pronounced among those with cyclin D1+ tumors than among those with cyclin D1− tumors. For other gastric adenocarcinomas, this difference was more pronounced, with a slight reduction in the OR for cyclin D1+ tumors (0.90; 95% CI =

*Table 3* Adjusted[a] odds ratios (OR) and 95% confidence intervals (CI) for cyclin D1 positive (+) and cyclin D1 negative (−) esophageal and gastric cancer in relation to use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) by tumor type among men and women in Connecticut, New Jersey, and Washington, 1993–1995

| Tumor type | Controls ($N$ = 692) | Cyclin D1+ cases | | Cyclin D1− cases | | Ratio of the ORs (95% CI) |
|---|---|---|---|---|---|---|
| | | $N$ = 279 | OR (95% CI) | $N$ = 338 | OR (95% CI) | |
| All esophageal and gastric cancers | | | | | | |
| NSAID use | | | | | | |
| Never | 410 | 192 | 1.0 | 223 | 1.0 | |
| Any type | 282 | 87 | 0.62 (0.45, 0.84) | 115 | 0.68 (0.51, 0.91) | 0.91 (0.64, 1.29) |
| Aspirin only | 222 | 73 | 0.66 (0.47, 0.92) | 91 | 0.67 (0.49, 0.92) | 0.98 (0.67, 1.43) |
| Esophageal adenocarcinoma | | | | | | |
| NSAID use | | | | | | |
| Never | 410 | 59 | 1.0 | 42 | 1.0 | |
| Any type | 282 | 21 | 0.45 (0.26, 0.79) | 33 | 1.12 (0.67, 1.86) | 0.40 (0.20, 0.82) |
| Aspirin only | 222 | 19 | 0.51 (0.28, .091) | 26 | 1.11 (0.64, 1.93) | 0.46 (0.22, 0.97) |
| Gastric cardia adenocarcinoma | | | | | | |
| NSAID use | | | | | | |
| Never | 410 | 34 | 1.0 | 54 | 1.0 | |
| Any type | 282 | 19 | 0.78 (0.43, 1.43) | 39 | 1.01 (0.63, 1.61) | 0.77 (0.38, 1.59) |
| Aspirin only | 222 | 15 | 0.80 (0.41, 1.53) | 31 | 1.05 (0.63, 1.74) | 0.75 (0.35, 1.65) |
| Esophageal squamous cell carcinoma | | | | | | |
| NSAID use | | | | | | |
| Never | 410 | 57 | 1.0 | 20 | 1.0 | |
| Any type[a] | 282 | 30 | 0.66 (0.39, 1.12) | 5 | 0.30 (0.10, 0.84) | 2.29 (0.73, 7.14) |
| Aspirin only | 222 | 24 | 0.68 (0.38, 1.21) | 5 | 0.36 (0.12, 1.07) | 1.87 (0.58, 6.01) |
| Noncardia gastric adenocarcinomas | | | | | | |
| NSAID use | | | | | | |
| Never | 410 | 42 | 1.0 | 107 | 1.0 | |
| Any type[a] | 282 | 17 | 0.54 (0.29, 1.00) | 38 | 0.43 (0.27, 0.65) | 1.27 (0.62, 2.58) |
| Aspirin only | 222 | 15 | 0.58 (0.30, 1.12) | 29 | 0.39 (0.24, 0.63) | 1.50 (0.70, 3.21) |

[a] Adjusted for center, race, sex, use of any alcohol (beer, wine and hard liquor), and body mass index.

0.51, 1.61) and a more than doubling of effect for cyclin D1− tumors (2.37, 95% CI = 1.50, 3.74); this heterogeneity was significant (ratio of the ORs for cyclin D1+ tumors/cyclin D1− tumors = 0.38; 95% CI = 0.19, 0.75).

The ORs for esophageal and gastric cancers associated with ever use of NSAIDs were generally decreased for both cyclin D1+ tumors and cyclin D1− tumors. Again, however, the pattern varied by the tumor subtype, as shown in Table 3. For esophageal adenocarcinoma, the ORs were reduced for tumors that overexpressed cyclin D1 (0.45; 95% CI = 0.26, 0.79) but not for tumors that did not (1.12; 95% CI = 0.67, 1.86). This heterogeneity was significant (ratio of the ORs for cyclin D1+ tumors to cyclin D1− tumors = 0.40; 95% CI = 0.20, 0.82). For gastric cardia adenocarcinoma, a similar but less pronounced pattern was noted, with a modest decrease in the OR for cyclin D1+ tumors and little or no increase for cyclin D1− tumors. However, for esophageal squamous carcinoma and for other gastric adenocarcinomas, there was a reduction in the OR for both cyclin D1+ tumors and cyclin D1− tumors, with a somewhat larger decrease among those with cyclin D1− tumors.

The pattern of associations observed for current aspirin use in relation to esophageal and gastric cancers stratified by cyclin D1 status (data not shown) reflected those observed for ever use shown in Table 3. However, the number of subjects who reported current use was lower than those reporting ever use; thus, the CIs were wider and the results less stable. The prevalence of use of other prescription medications (such as $H_2$ receptor antagonists) among our study subjects was very low (12, 13), prohibiting evaluation of whether their relation to esophageal and gastric cancer varied with cyclin D1. Regarding the associations between use of over-the-counter antacids and esophageal and gastric cancer, there was no heterogeneity with cyclin D1 (data not shown). Furthermore, the ORs for esophageal and gastric cancers in relation to body size or any alcohol intake (including beer, wine, and hard liquor considered separately) did not vary with the presence or absence of cyclin D1 overexpression (data not shown).

## Discussion

This is the first population-based study to report on the prevalence of cyclin D1 status assessed by immunohistochemistry in the tumor tissue of esophageal and gastric cancers. Overexpression of cyclin D1 was noted in over three-quarters of patients with esophageal squamous cell carcinoma, over half of patients with esophageal adenocarcinoma, over a third of patients with gastric cardia adenocarcinoma, and more than a quarter of patients with other gastric adenocarcinomas. These results are consistent with other estimates that were based on hospital-based or clinical surveys of patients (2).

This epidemiological report is also the first to examine whether the risk for these cancers in humans varies with cyclin D1 expression in relation to NSAID use, cigarette smoking, and other factors. A study in laboratory animals (18) found that subcutaneous administration of *N*-nitrosomethylbenyzlamine, which is a constituent of tobacco smoke, increased the severity of esophageal squamous dysplasia and cellular proliferation in transgenic mice that overexpress cyclin D1. Our results are not consistent with this laboratory finding. We observed no substantial difference in the ORs associated with cigarette smoking among those with cyclin D1+ tumors *versus* those with cyclin D1− tumors. Regardless of cyclin D1 status, cigarette smoking was associated with an elevated risk of esophageal adenocarcinoma, gastric cardia adenocarcinoma, and esophageal squamous cell carcinoma. For other gastric adenocarcinomas, only

tumors that did not overexpress cyclin D1 had an elevated OR, especially among current smokers. Similarly, little heterogeneity of effect was noted for several other factors in relation to esophageal and gastric cancer, including obesity, alcohol intake, or antacid use.

In the data reported here, the decrease in adenocarcinomas of the esophagus and gastric cardia associated with aspirin or NSAID use was observed only for subjects whose tumors overexpressed cyclin D1, and no effect was observed among tumors that did not overexpress cyclin D1. The precise mechanism by which NSAIDs reduce cancer risk has not been fully elucidated (8, 19). Aspirin has been shown *in vivo* to inhibit COX1 and COX2, thereby blocking the production of prostaglandins but has little effect on these activities *in vitro*; also, the biochemical effects of salicylate appear to be independent of effects on COX activity (20). Thus other mechanisms of action are under consideration. For example, sulindac, sulindac sulfide, and other NSAIDs have been shown to inhibit proliferation and induce apoptosis in colon cancer cells, consequences that have been attributed to their effect on the cell cycle (21). Among the important cell cycle-regulators are the cyclins and the cyclin-dependent kinases (1). In laboratory studies, cyclin D1 expression was reduced by aspirin and by indomethacin in four colorectal cell lines (22), decreased by sulindac sulfide in colon cancer cells (23) and in breast cancer cell lines (24), and inhibited by sodium salicylate in pancreatic cell lines (25).

Our observations, if corroborated in other studies, may help to identify patients that may particularly benefit from intervention with NSAIDs. It is noteworthy that the risk of esophageal adenocarcinoma is much greater among patients with Barrett's esophagus that show evidence of cyclin D1 overexpression than those without cyclin D1 overexpression (5). Thus, it is plausible that the chemopreventive effects of NSAIDs may be more favorable when lesions of Barrett's esophagus overexpress cyclin D1. Other observations among patients with Barrett's esophagus are consistent with this idea; in a cross-sectional study, Vaughan *et al.* (26) noted that, among Barrett's patients, NSAID use was not associated with loss of heterozygosity of 9p allele (which would knock out p16), but was strongly associated with aneuploidy, increased 4N (tetraploidy) fraction, 17p loss of heterozygosity, and high-grade dysplasia. Given the complex interplay between p16, cyclins, and cyclin-dependent kinases, these observations are consistent with the likely implications of our findings that NSAID use may reduce the progression of Barrett's esophagus to esophageal adenocarcinoma and may be most effective among Barrett's patients with lesions that overexpress cyclin D1+.

Our findings, however, must be cautiously interpreted. Even in the most carefully conducted epidemiological study, bias can arise at several steps, including the inclusion of subjects who choose to participate, the difficulty in recalling historical behaviors or events, as well as the proportion of patients for whom archived block tissue was available and usable. The molecular epidemiology study reported here was built on a parent study that was population-based with a reasonable response rate (9), which reduces the likelihood of bias arising because of sample selection. In Table 1, we were able to demonstrate that the proportion of patients for whom we had archived tissue that was usable for these analyses was not systematically different from the entire sample of case participants. It is possible that cases and controls may have differentially recalled their use of medications, such as aspirin or other NSAIDs. Heterogeneity with cyclin D1 status, however, was not evident for the reported use of over-the-counter antacids, suggesting that the heterogeneity noted for NSAID use was not a spurious result. Furthermore, for recall to influence the results reported here, case response to the comprehensive questionnaire would have had to have been reported differentially based on their cyclin D1 status, which is very unlikely (27).

In summary, the results of our population-based study confirm the high prevalence of cyclin D1 overexpression in various histological subgroups of esophageal and gastric tumors. In determining whether risk factors for these cancers varied by the presence or absence of cyclin D1 overexpression, we found little variation for alcohol intake, body size, or cigarette smoking, except that the risk of noncardia gastric adenocarcinomas was not elevated for tumors that were cyclin D1+. In contrast, the reduced risk for esophageal and gastric cardia adenocarcinomas associated with the use of aspirin or other NSAIDs was restricted to patients with tumors that overexpressed cyclin D1. Because cyclin D1 is overexpressed in lesions of Barrett's esophagus that progress to esophageal adenocarcinoma (4, 5), our data, if replicated, suggest that targeting these patients for intervention with NSAIDs may be particularly fruitful.

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