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Research Article

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon with a high incidence of colon cancer. Dysplasia is a precursor to carcinoma and a predictor of malignant potential; epithelia containing high-grade or severe dysplasia is most likely to develop cancer. The cellular oncogene c-src and its viral homologue v-src (the transforming gene of Rous sarcoma virus) encode 60-kD cytoplasmic, membrane-associated protein tyrosine kinases. For the viral protein or transforming mutants of the cellular protein (Src), a close correlation exists between elevated tyrosine kinase activity and malignant transformation of cells. Previously, we and others observed elevated Src activity in sporadic colon carcinomas and benign adenomas at greatest risk for developing cancer (those with large size, villous architecture, and/or severe dysplasia). Here we report that Src activity and protein abundance are also elevated in neoplastic UC epithelia. Activity is highest in malignant and severely dysplastic epithelia, and 6-10-fold higher in mildly dysplastic than in nondysplastic epithelia. Thus, Src activity is elevated in premalignant UC epithelia, which is at greatest risk for developing cancer. The data suggest that activation of the src proto-oncogene is an early event in the genesis of UC colon cancer.

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Elevated c-Src Tyrosine Kinase Activity in Premalignant Epithelia of Ulcerative Colitis

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Abstract

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon with a high incidence of colon cancer. Dysplasia is a precursor to carcinoma and a predictor of malignant potential; epithelia containing high-grade or severe dysplasia is most likely to develop cancer. The cellular oncogene *c-src* and its viral homologue *v-src* (the transforming gene of Rous sarcoma virus) encode 60-kD cytoplasmic, membrane-associated protein tyrosine kinases. For the viral protein or transforming mutants of the cellular protein (Src), a close correlation exists between elevated tyrosine kinase activity and malignant transformation of cells. Previously, we and others observed elevated Src activity in sporadic colon carcinomas and benign adenomas at greatest risk for developing cancer (those with large size, villous architecture, and/or severe dysplasia). Here we report that Src activity and protein abundance are also elevated in neoplastic UC epithelia. Activity is highest in malignant and severely dysplastic epithelia, and 6–10-fold higher in mildly dysplastic than in nondysplastic epithelia. Thus, Src activity is elevated in premalignant UC epithelia, which is at greatest risk for developing cancer. The data suggest that activation of the *src* proto-oncogene is an early event in the genesis of UC colon cancer. (*J. Clin. Invest.* 1994. 93:509–515.) Key words: oncogene • protein kinase • colon cancer • dysplasia

Introduction

Colon carcinoma is the most common gastrointestinal cancer and the second most common cancer overall in the United States (1). The estimated 152,000 new cases and 57,000 deaths from the disease during 1993 alone illustrate its impact on cancer-associated morbidity and mortality. The risk of colon cancer is particularly high in ulcerative colitis (UC),¹ a chronic inflammatory disease of the colon. The cumulative risk is 0.5–1%/yr after the first 10 yr of disease (e.g., 10–20% after 30 yr of colitis involving the entire colon) and the relative risk is 15-fold higher than expected in age-matched populations without colitis (2, 3).

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1. Abbreviations used in this paper: DALM, high-grade dysplasia-associated lesion or mass; HGD, high-grade dysplasia; LGD, low-grade dysplasia; UC, ulcerative colitis.

The cellular oncogene *c-src* and its viral homologue *v-src* (the transforming gene of Rous sarcoma virus) encode 60-kD cytoplasmic, membrane-associated, protein tyrosine kinases (4–8). For the viral protein (*v-Src*) or transforming mutants of the cellular protein (Src), a close correlation exists between elevated tyrosine kinase activity and malignant transformation of cells. Src activity is elevated in three human cancers: colon and breast adenocarcinomas, and neuroblastoma (9–12). Previously, we observed that Src from 13 of 21 primary colon carcinomas had 5–7-fold higher activity than Src from normal adjacent mucosa. Similarly, Src from 6 of 9 colon carcinoma cell lines had 5–20-fold higher activity than that from normal colonic cells (12). Moreover, only those colon carcinoma cell lines with elevated Src activity had elevated levels of three phosphotyrosine-containing proteins, thereby suggesting a functional correlation for the increased Src tyrosine kinase activity. We also observed increased Src activity in colonic adenomas containing focal carcinoma, and in benign adenomas at greatest risk for developing cancer (those with large size, villous architecture, and/or severe dysplasia) (13). Interestingly, segments of fetal rat colon infected with a *v-src*-containing retrovirus and implanted in syngeneic pups developed significant dysplasia throughout the colonic epithelia (14).

Dysplasia is a precursor to carcinoma in adenomas and in UC. To determine whether Src activity is elevated in precancerous UC tissue as it is in adenomas, we measured its kinase activity in normal, acute or chronically inflamed, dysplastic and malignant epithelia from 35 UC patients. Src activity in malignant or dysplastic epithelia was significantly higher than that in nondysplastic epithelia from the same individual. Moreover, the level of Src activity in dysplastic epithelia correlated with the degree of dysplasia: the more severe the dysplasia, the higher the Src activity.

Methods

Patients and tissue collection. Colonic mucosal samples were obtained from individuals with UC for ≥ 10 yr. Specimens were collected during colectomy or colonoscopy for cancer surveillance. Mucosal samples were obtained at 10-cm intervals throughout the colon and divided into two parts. One part was formalin fixed and analyzed microscopically, the other part was frozen in liquid nitrogen (within 1 min if collected during colonoscopy; within 10 min if collected surgically) and analyzed for Src protein levels and tyrosine kinase activity. The protocol was approved by the institutional administrative panel on human subjects in medical research, and informed written consent was obtained from all patients.

Histopathology. Specimens were independently analyzed by two staff pathologists. Both pathologists were blinded to the Src kinase assay results. Pathologic classifications were those of Riddell et al. (15). Definitions used are: mild quiescent colitis: increased numbers of lymphocytes, plasma cells, eosinophils, and macrophages in the lamina propria; severe quiescent colitis: dense mononuclear cell infiltrates (predominantly lymphocytes and plasma cells), distorted crypt architec-

ture with branched or irregular glands, crypt atrophy, and goblet cell mucin depletion; active colitis: polymorphonuclear cell infiltration into the lamina propria, often with crypt injury (cryptitis, crypt ulcer, or crypt abscess), epithelial destruction, and concurrent regeneration; chronic active colitis: mixed active colitis, destruction, regeneration, and resolving colitis; dysplasia: neoplastic epithelial proliferation with enlarged irregular hyperchromatic crowded and stratified nuclei extending halfway (mild or low-grade dysplasia [LGD]), or more than halfway (severe or high-grade dysplasia [HGD]), toward the luminal surface of the cell; invasive adenocarcinoma: malignant glandular epithelium extending through the muscularis mucosae. With the exception of patient 5, who had HGD or carcinoma in situ in a cecal mass, both pathologists agreed on histopathologic diagnoses. Of note, many morphologists use the term HGD synonymously with carcinoma in situ to indicate the malignant nature of these cells (15).

Protein extractions, immunoprecipitations, and tyrosine kinase assays. Tissues were homogenized in Kontes glass tissue grinders and cells were lysed in ice-cold, modified RIPA buffer (0.1% SDS, 1% NP-40, 1% sodium deoxycholate, 0.15 M sodium chloride, 10 mM sodium phosphate [pH 7.0], 100 μ M sodium vanadate, 50 mM sodium fluoride, 50 μ M leupeptin, 1% aprotinin, 2 mM EDTA, and 1 mM dithiothreitol). Lysates were centrifuged at 29,000 g for 1 h at 4°C and protein concentrations were measured by the BCA protein assay (Pierce, Rockford, IL). Lysate containing 90 μ g protein was incubated with excess mAb specific for Src (mAb 327) (16), or with rabbit anti-peptide antibody specific for a Src-related tyrosine kinase, c-Yes (17). Excess affinity-purified rabbit anti-mouse immunoglobulin G (Cooper Biomedical, Malvern, PA) was added to mAb 327 precipitates. For in vitro protein-tyrosine kinase reactions (18–22), immunoprecipitates were incubated for 15 min at 30°C in 30 μ l of buffer containing 50 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (pH 7.0), 10 mM manganese chloride, 10 mM dithiothreitol, 1 μ g acid-denatured rabbit muscle enolase (23) (Boehringer Mannheim Biochemicals, Indianapolis, IN), and 50 μ Ci of γ -[³²P]ATP (4,000 Ci/mmol; ICN, Costa Mesa, CA). Adding unlabeled ATP (20 μ M) and/or incubating samples for 10 min gave similar results. c-Src in vitro protein kinase activity is linearly related to the concentration of total cellular protein (12, 13). Proteins were resolved by 7% SDS-PAGE and gels were stained with Coomassie brilliant blue G-250 (Bio-Rad Laboratories, Richmond, CA) to confirm that equivalent amounts of enolase were present in each lane. Radiolabeled proteins were detected using Kodak XAR film and an intensifying screen at –80°C. ³²P incorporation into proteins was quantified by Cerenkov counting. Quantitation by liquid scintillation spectroscopy after gel pieces are dissolved in 1.2 ml of 30% H₂O₂/70% perchloric acid (2:1) at 55°C for 12 h gives similar results (12, 13).

Immunoblot analysis. mAb 327 immunoprecipitates, prepared in parallel with those used for in vitro protein kinase assays, were resolved by 7% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (0.45 μ m; Schleicher & Schuell, Inc., Keene, NH) in transfer buffer (12.5 mM Tris-HCl [pH 8.3], 100 mM glycine, and 20% methanol) using a Trans-Blot apparatus (Bio-Rad Laboratories) for 4 h at 40 V (12, 13). Membranes were incubated overnight in blocking buffer containing TNE (10 mM Tris-HCl [pH 7.5], 50 mM NaCl, 2.5 mM EDTA, and 0.01% NaN₃), 0.1% (vol/vol) Tween 20 (Sigma Chemical Co., St. Louis, MO), and 5% nonfat powdered milk. After blocking, membranes were incubated for 2 h with normal sheep serum (Cooper Biomedical) (1:10 dilution in blocking buffer), then for 1 h with puri-

fied mAb 327 ascites (1:250 dilution in blocking buffer) at 22°C. Under these conditions antibody was in excess of antigen. Membranes were washed in TNE buffer with changes every 10 min for 1 h before incubating with excess ¹²⁵I-labeled affinity-purified, sheep anti-mouse IgG (New England Nuclear, Boston, MA) (10⁶ cpm/ml blocking buffer) for 1 h at 22°C. After washing in TNE buffer for 1 h, ¹²⁵I-labeled proteins were visualized by autoradiography with an intensifying screen at –80°C, and quantified by counting excised bands. The amount of Src protein detected in lysates is linear over a fivefold range of total cellular protein (12, 13).

Results

To determine whether Src activity is elevated in dysplastic UC epithelia as it is in dysplastic polyps, we collected colonic mucosal samples from 35 UC patients and analyzed tissue for histopathology (Table I) and Src in vitro protein-tyrosine kinase activity (Figs. 1–5). 8 patients had 27 samples of normal epithelia proximal to the involved UC epithelia, 16 had 39 samples of active colitis, 27 had 118 samples of mild quiescent colitis, 8 had 21 samples of severe quiescent colitis, 4 had 10 samples of LGD, 3 had 11 samples HGD, and 2 had invasive carcinoma. Src activity measured in samples from seven representative patients is shown:

Src tyrosine kinase activity in active or quiescent UC. Patient 1 (Fig. 1 A) had left-sided, distal UC involving the recto-sigmoid colon for \geq 13 yr. Normal epithelia was present in the proximal colon (ascending, transverse, and descending colon), mild quiescent colitis in the sigmoid colon, and moderate-to-severe active colitis in the rectum. Src kinase activity varied little between different regions of normal epithelium (compare lanes 1–4). Moreover, Src activity varied little between normal epithelia and that containing mild quiescent colitis (compare lanes 1–4 with lane 5), or that containing moderate-to-severe active colitis (compare lanes 1–4 with lane 6). Thus, in this individual and others, Src activity in active or mild quiescent colitis was similar to that in normal epithelia.

Patient 2 (Fig. 1 B) had universal UC (involving the entire colon) for 13 yr. The ascending, transverse, descending, and proximal sigmoid colon contained mild quiescent colitis. The distal sigmoid colon and rectum contained severe quiescent colitis. Src activity varied little between different regions of mild quiescent colitis (compare lanes 1–5). In contrast, Src activity in severe quiescent colitis (lanes 6 or 7) was two- to threefold higher than that in mild quiescent colitis (lanes 1–5).

Src tyrosine kinase activity in dysplastic UC epithelia. Patient 3 (Fig. 2 A) had universal UC for 11 yr. Severe quiescent colitis was present throughout the colon. In addition, mild dysplasia or LGD was present in the right colon (cecum, ascending, and proximal transverse colon). Src activity in the dysplastic epithelia (lanes 1–3) was, on average, threefold higher as measured by enolase phosphorylation or fivefold higher as measured by autophosphorylation than Src activity in the non-dysplastic severe quiescent colitis (lanes 4–8).

Table I. Histopathology of Colonic Mucosa from UC Patients

	Normal	Active colitis	Quiescent (mild)	Quiescent (severe)	LGD	HGD	Cancer
Patients	8	16	27	8	4	3	2
Samples	27	39	118	21	10	11	2

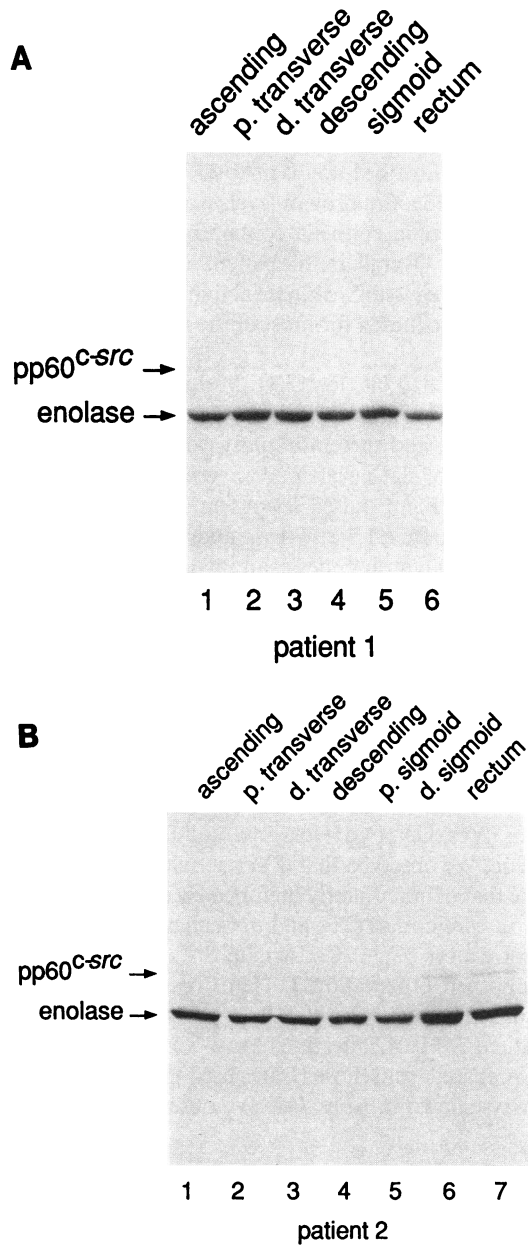


Figure 1. Src tyrosine kinase activity in active or quiescent UC. (A) Patient 1: lanes 1–4, normal epithelia; lane 5, mild quiescent colitis; lane 6, moderate-to-severe active colitis. (B) Patient 2: lanes 1–5, mild quiescent colitis; lanes 6 and 7, severe quiescent colitis. Mucosal samples were homogenized and cell lysates containing 90 μ g of protein were prepared. Proteins were immunoprecipitated with excess mAb 327, incubated with γ -[³²P]ATP and enolase, and resolved by SDS-PAGE. Autoradiogram exposure times at -80°C with intensifying screens: (A) 20 min; (B) 15 min. pp60^{c-src}, c-Src; p., proximal; d., distal.

Patient 4 (Fig. 2 B) had universal UC for 26 yr. Mild quiescent colitis was present throughout the colon. In addition, LGD was present in the rectal epithelium and in a small, rectal adenoma. Src activity in either region containing LGD (lane 2 or 3) was five- or sevenfold higher (as measured by enolase or autophosphorylation, respectively) than Src activity in the nondysplastic quiescent colitis (lane 1). A phosphorylated protein with an approximate molecular mass of 65–70 kD (arrowhead) was detected in lanes 2 and 3. This protein was reproducibly

detected in dysplastic epithelia from this patient, and rarely in others. Its identity is being investigated.

Patient 5 (Fig. 2 B) had universal UC for 19 yr. The entire colon contained mild quiescent colitis. In addition, a large mass measuring 5 cm in diameter was present in the cecum. Six biopsies of the lesion all showed HGD as read by one pathologist or carcinoma in situ as read by the second pathologist. Src activity in the mass (lane 5) was 23- or 21-fold higher than that in adjacent nondysplastic quiescent colitis (lane 4). After surgical resection of the cecal mass, HGD or carcinoma in situ was again found throughout the lesion.

Src tyrosine kinase activity in UC carcinoma. Patient 6 (Fig. 3) had universal UC for 16 yr. All biopsies obtained at colonoscopy and later at colectomy contained dysplasia. LGD

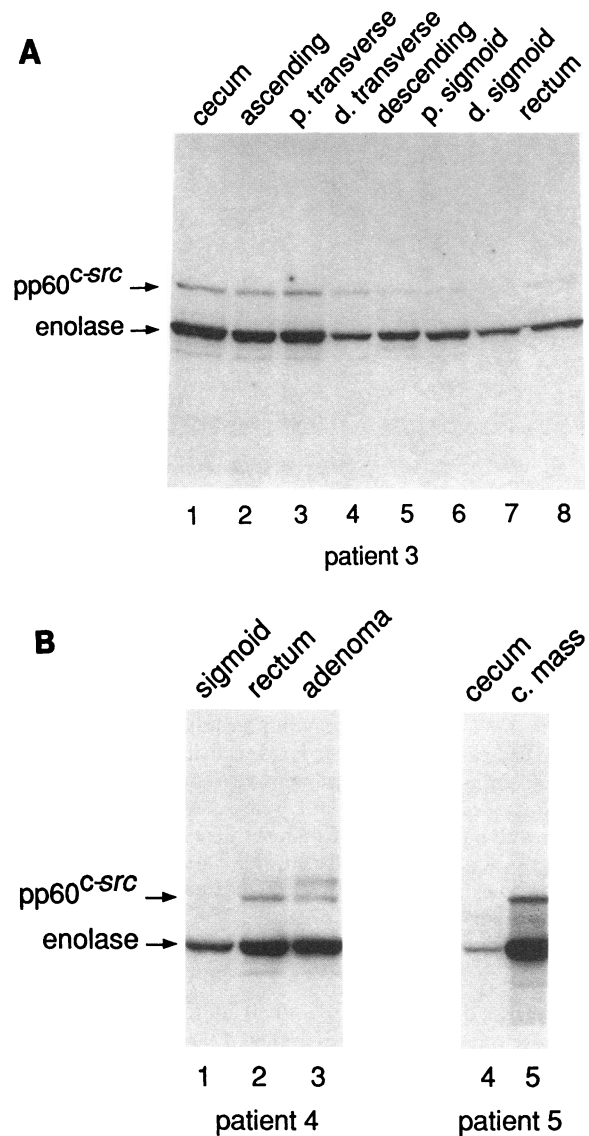


Figure 2. Src tyrosine kinase activity in dysplastic UC epithelia. (A) Patient 3: lanes 1–3, LGD; lanes 4–8, severe quiescent colitis. (B) Patient 4: lane 1, mild quiescent colitis; lanes 2 and 3, LGD. Patient 5: lane 4, mild quiescent colitis; lane 5, HGD or carcinoma in situ. mAb 327 immune complex protein kinase reactions were performed as described in the legend to Fig. 1. Exposure times at -80°C with intensifying screens: (A) 18 min; (B) lanes 1–3, 10 min; lanes 4 and 5, 20 min. p., proximal; d., distal; c., cecal.

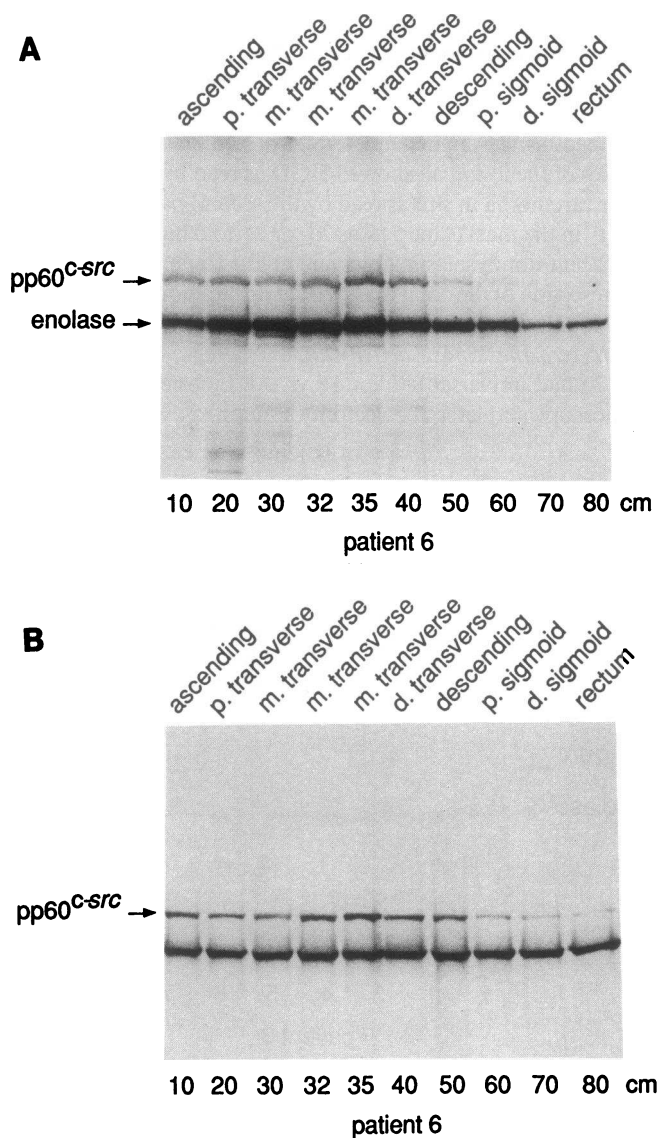


Figure 3. Src tyrosine kinase activity and protein levels in UC carcinoma. Patient 6: 10–60 cm (distance from cecum), HGD; 70–80 cm, LGD; 35 cm, carcinoma. (A) Src kinase activity. (B) Src protein levels. mAb 327 immunoprecipitates, prepared from lysates used for the protein kinase reactions shown in A, were resolved by SDS-PAGE and transferred to nitrocellulose. Membranes were incubated with excess mAb 327, washed, and incubated with ¹²⁵I-sheep anti-mouse IgG. Exposure times at –80°C with intensifying screens: (A) 40 min; (B) 10 h. *p.*, proximal; *m.*, mid; *d.*, distal.

was present in the distal colon (70 and 80 cm from the cecum), and HGD was present in the remainder of the colon. In addition, a sessile 1.5-cm polypoid lesion in the transverse colon (35 cm), and 1 of 96 lymph nodes examined, contained moderately differentiated adenocarcinoma. The tumor stage was C1 by the Astler-Coller modification of the Dukes' classification (24, 25). Src activity (A) in epithelia containing HGD was higher (on average, fourfold as measured by enolase phosphorylation, or sixfold as measured by autophosphorylation) than Src activity in epithelia containing LGD. Src activity in the carcinoma was 6- or 10-fold higher than that in epithelia containing LGD, and ~ 2-fold higher than that in epithelia containing HGD.

Src protein levels (Fig. 3 B) were determined by immunoblot analysis. mAb 327 immunoprecipitates, performed in parallel with those used for the kinase assays shown in Fig. 3 A, were resolved by SDS-PAGE before being transferred to a nitrocellulose membrane. Two proteins were detected in each lane: the upper one is Src, the lower is mAb 327 immunoglobulin heavy chain (12, 13). Src protein levels were highest in the carcinoma, and higher in epithelia containing HGD than in that containing LGD. Overall, in this patient and in others, Src protein levels correlated with Src kinase activity, and increased protein expression accounted for most of the increased kinase activity.

Patient 7 (Fig. 4) had UC for 15 yr. Multiple biopsies obtained at colonoscopy, and later at colectomy, contained chronic active colitis, and therefore, most were indefinite for dysplasia. A region of flat cecal mucosa was suspicious for HGD, and two, ≤ 0.5 cm, raised lesions in the cecum contained HGD. In addition, a 1.5-cm, firm, nodular lesion in the cecum, and 1 of > 50 lymph nodes examined, contained mucinous adenocarcinoma (Duke's C1). Src activity in either of the high-grade dysplasia-associated lesions or masses (DALMs) was threefold higher than that in flat mucosa containing HGD (compare lanes 2 or 3 with lane 4). Src activity in the carcinoma was five- or sevenfold higher (as measured by autophosphorylation or enolase phosphorylation, respectively) than that in HGD (compare lanes 1 and 4).

Recently, we examined the tyrosine kinase activity of Src-related proteins (Yes, Lck, Fyn, Hck, Lyn, and Fgr) in human colon carcinoma. We observed that c-Yes activity, like that of Src, and unlike that of other family members, was significantly elevated in most carcinomas (26) and in benign adenomas at greatest risk for cancer (C. A. Cartwright, S. Pena, and A. I. Meisler, unpublished observations). Here we measured the protein-tyrosine kinase activity of Yes in the neoplastic UC epithelia of patient 7 (Fig. 4, lanes 5–8). Anti-Yes immunoprecipitates were prepared from those lysates used for the Src protein kinase assays shown in lanes 1–4. We observed that Yes

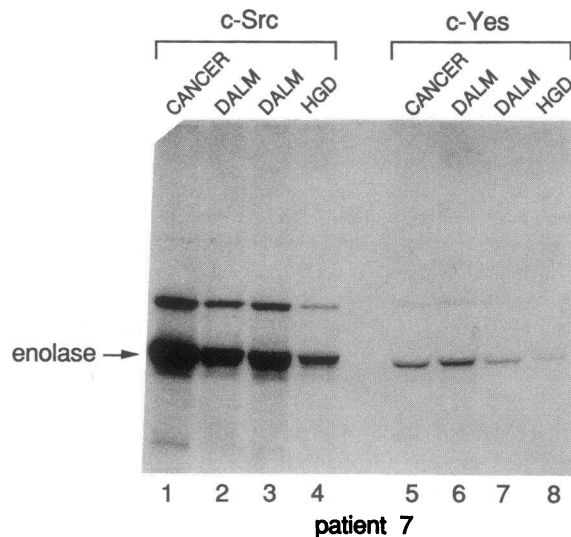


Figure 4. Src and Yes tyrosine kinase activity in UC carcinoma. Patient 7: lanes 1 and 5, carcinoma; lanes 2, 3, 6, and 7, DALM; lanes 4 and 8, HGD in flat epithelia. Exposure time at –80°C with an intensifying screen: 55 min.

activity, similar to that of Src, was higher in carcinoma and in DALMs than in flat dysplastic epithelia (compare lanes 5–7 with lane 8). As predicted from its molecular mass, Yes (62 kD) migrated more slowly than Src (60 kD) in the gel. Overall, Src activity was higher than that of Yes in the same epithelia (compare lanes 1–4 with 5–8).

Relative levels of Src tyrosine kinase activity in UC epithelia. Data on Src activity in colonic epithelia from 35 UC patients are summarized in Fig. 5. Values from two to four independent measurements of Src activity were averaged for each sample, and values from different regions of the colon that contained the same histopathology were averaged for each patient. Values shown represent the mean±SEM for patients within each group.

Data from those patients who had regions of mild quiescent colitis are shown in Fig. 5 A. Values are expressed relative to those for mild quiescent colitis. Src activity in normal epithelia or in active colitis was similar to that in mild quiescent colitis.

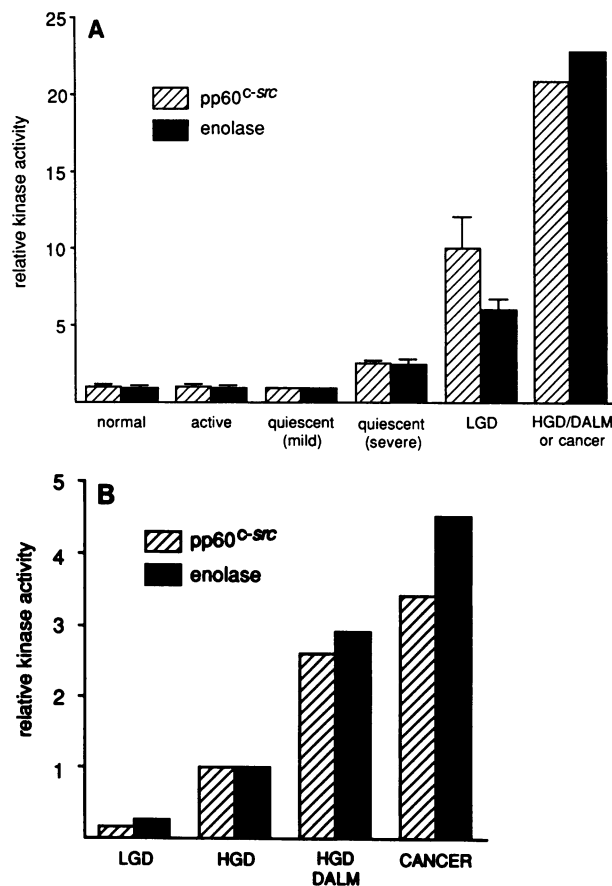


Figure 5. Relative levels of Src tyrosine kinase activity in UC epithelia. (A) UC patients whose colons contained regions of mild quiescent colitis. LGD, HGD/DALM, or carcinoma in situ (cancer). (B) UC patients whose colons contained LGD, HGD, HGD/DALM, and/or invasive carcinoma. ³²P incorporation into Src or enolase was quantified by counting excised gel bands. Values from two to four independent measurements of Src activity were averaged for each sample, and values from different regions of the colon that contained the same histopathology were averaged for each patient. Values shown represent the mean±SEM for patients within each group, and are expressed relative to those for mild quiescent colitis (A) or HGD (B). (Hatched bars) Src autophosphorylation; (solid bars) enolase phosphorylation.

In contrast, Src activity in severe quiescent colitis was higher (2.6-fold as measured by autophosphorylation, or 2.5-fold as measured by enolase phosphorylation) than that in mild quiescent colitis. Src activity in LGD was 10- or 6.1-fold higher, and that in HGD/DALM or carcinoma in situ was 20.9- or 22.8-fold higher than that in mild quiescent colitis. Thus, Src activity is elevated in dysplastic UC epithelia. In addition, Src activity in nondysplastic severe chronic colitis is significantly higher than that in mild chronic or active colitis, or normal epithelia.

Data from two additional UC patients who had LGD, HGD, DALMs, and carcinomas are summarized in Fig. 5 B. Because these two patients had no mild quiescent colitis for comparison, their data could not be analyzed with those shown in Fig. 5 A. Values are expressed relative to those for HGD. Src activity in HGD was 6.3- or 3.9-fold higher (as measured by autophosphorylation or enolase phosphorylation, respectively) than that in LGD. Src activity in HGD/DALMs was 2.6- or 2.9-fold higher than that in HGD in flat epithelia. Finally, Src activity in carcinomas was 3.4- or 4.5-fold higher than that in HGD. Thus, the level of Src activity correlates with the degree of dysplasia: the more severe the dysplasia, the higher the Src activity. Moreover, Src activity in HGD-associated lesions is intermediate between that in invasive carcinoma and that in HGD in flat epithelia.

Six patients had active colitis that was indefinite for dysplasia at year 1, and inactive quiescent colitis that was negative for dysplasia in the same regions of the colon on follow-up evaluation at year 2 (and for two patients, again at year 3). In all six patients, Src activity measured in the active colitis at year 1 was very similar to that measured in the inactive colitis in the same region at year 2 (and for the two patients, again at year 3). Thus, Src activity remained constant in epithelia that initially were indefinite for dysplasia because of active colitis, and later, when the colitis was quiescent, were clearly and reproducibly negative for dysplasia.

Discussion

The data show that Src tyrosine kinase activity and protein abundance are elevated in neoplastic UC epithelia. Activity is highest in malignant and severely dysplastic epithelia, and 6–10-fold higher in mildly dysplastic than in nondysplastic epithelia. Thus, Src activity is elevated in premalignant UC epithelia, which is at greatest risk for developing cancer. The data suggest that activation of the *src* proto-oncogene is an early event in the genesis of UC colon cancer.

Several groups have identified genes whose structure, expression, or activity is altered in different stages of sporadic colon cancer (27). A number of genes are affected relatively late in tumorigenesis, i.e., in carcinomas. These include the putative tumor suppressor genes p53 and DCC (28–30). Other genes, like the dominant acting oncogene *K-ras*, seem to be altered earlier in tumorigenesis, in adenomas with a high malignant potential (31). We have shown that the Src and Yes tyrosine kinase activities and protein levels are elevated both in carcinomas and in adenomas (12, 13, 26). Expression of members of the *myc* gene family is abnormal in essentially all adenomas, independent of their malignant potential (32, 33). Mutations of the APC locus may represent one of the earliest events in the genesis of colon cancer (34–38).

Src and *myc* are overexpressed in many of the same colon carcinomas and adenomas (12, 13, 32, 33). Interestingly, seg-

ments of fetal rat colon infected with a *myc*-containing retrovirus and implanted in syngeneic pups develop epithelial atypia and focal dysplasia (14). Segments infected with a *v-src*-containing retrovirus develop more significant dysplasia throughout the colonic epithelia. Moreover, the segments infected with both oncogenic retroviruses together develop HGD and in one case adenocarcinoma. Thus, *myc* and *src* may act synergistically to induce dysplasia.

Genetic alterations in premalignant UC epithelia include p53 point mutations and loss of heterozygosity (39, 40), *Myc* (41), and *Src* overexpression, and, rarely, *ras* mutations (42–45). p53 inactivation and *Src* overexpression occur earlier in the histologic progression of neoplasia in UC than in sporadic colon cancer. These observations suggest that the etiology and pathogenesis of sporadic and UC-associated carcinogenesis may differ in significant ways.

Colonic mucosa consists of epithelia and lamina propria. Normally the lamina propria contains lymphocytes, plasma and mast cells, macrophages, eosinophils, and fibroblasts. In quiescent UC, mononuclear cells (predominantly lymphocytes and plasma cells) infiltrate the lamina propria. It is possible that the increased *Src* activity measured in severe quiescent colitis is actually *Src* activity measured in these infiltrating cells. However, lymphocytes and plasma cells do not express *Src*, and macrophages express it at very low levels (46, 47). Therefore, it seems unlikely that these cells are contributing significantly to the *Src* activity in severe quiescent colitis. The cell population in the lamina propria of dysplastic mucosa is not significantly different than that in nondysplastic mucosa, and thus, cannot explain the striking differences observed in *Src* activity between these mucosae. In active UC, polymorphonuclear cells infiltrate the lamina propria. Again, it is possible that the *Src* activity measured reflects that found in these acute inflammatory cells. Although the level of *Src* activity in neutrophils is not known, *Src* activity measured in active colitis is remarkably similar to that in normal mucosa or mild quiescent colitis (Fig. 5 A) despite the varied numbers and types of inflammatory cells present in these different mucosae.

Src protein kinase activity and abundance decrease as human colon carcinoma cell lines differentiate in response to sodium butyrate (48). Moreover, when human colon carcinoma cell lines are treated with herbimycin A (an ansamycin antibiotic that inhibits cellular transformation by retroviral tyrosine kinases) there is a dose-dependent, reversible inhibition of cell growth and a concomitant decrease in *Src* kinase activity and steady-state protein levels (49). We observed that cytoskeletal-associated *Src* activity decreases as primary intestinal crypt cells terminally differentiate into enterocytes (50). These results together with those showing elevated *Src* activity in dysplastic and malignant colonic cells suggest that downregulation of *Src* is important for differentiation and upregulation is important for growth and transformation of intestinal epithelial cells.

Possible mechanisms upregulating *Src* expression in neoplastic colonic epithelia include: protein stabilization, gene overexpression, and/or gene amplification. Preliminary studies indicate that *src* mRNA levels are higher in colon carcinomas than in normal adjacent epithelia (C. A. Cartwright, unpublished observations). Protein overexpression accounts for most but not all of the elevated *Src* activity in premalignant colonic epithelia. Potential mechanisms upregulating the specific activity of the *Src* kinase include: dephosphorylation of

Src at Tyr 527, subcellular localization of *Src* and its substrates, association of *Src* with other cellular proteins, and genetic mutation in *Src* (reviewed in reference 8). In cultured colon carcinoma cells, we do not detect decreased phosphorylation of *Src* at Tyr 527 (12). Preliminary studies indicate that a large fraction of *Src* and many tyrosine kinase substrates in colon carcinoma cells localize to cellular membranes (K.-Y. Lee and C. A. Cartwright, unpublished observations). Recently, we isolated an *Src*-associated protein from a λ gt11 expression library of human colon carcinoma cells (Z.-Y. Peng and C. A. Cartwright, unpublished observations). The protein may be important for regulating *Src* kinase activity.

The recent discovery of widespread mutations in short, repeated DNA sequences in colon cancer reveals a novel mechanism of carcinogenesis distinct from those involving activated oncogenes or inactivated tumor suppressor genes (51–53). The data suggest the presence of a defective DNA replication factor resulting in reduced fidelity for replication or repair. Clearly, multiple, complex molecular mechanisms are involved in colonic carcinogenesis. Activation of the *Src* tyrosine kinase may be only one of a number of factors contributing to malignant transformation and may not be sufficient in itself to transform cells. Nonetheless, the consistent and striking nature of its activation in malignant and premalignant UC epithelia, together with the strong historical correlate of elevated *Src* activity and cell transformation, suggest that activation of this kinase may be an important early event in the genesis of UC colon cancer.

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