PTP1B Contributes to the Oncogenic Properties of Colon Cancer Cells through Src Activation

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Abstract

Src-specific activity has been reported to be elevated in a high percentage of colon cancer cell lines and tumors, but the underlying mechanisms are largely unknown. In this study, we report that, in the seven cancer cell lines tested, Src-specific activity was elevated (5.2- to 18.7-fold) relative to normal colon cells (FHC). This activation of Src correlated with reduced phosphorylation at Y530 of Src, whereas there was no significant change in the level of phosphorylation at Y419. The membrane tyrosine phosphatase activity for a Src family-specific phosphopeptide substrate FCP (Fyn COOH-terminal peptide phosphorylated by Csk) was greatly increased in the cancer cells and was attributed to PTP1B in most of the cell lines. Membrane PTP1B protein levels were also greatly increased. Overexpression of PTP1B increased Src specific activity in colon cancer cells by reducing phosphorylation at Y530 of Src. It also increased anchorage-independent cell growth and this increase was blocked by the Src inhibitor PP2 and Src small interfering RNA (siRNA). Down-regulating PTP1B activity by PTP1B inhibitor CinnGEL 2Me or knocking down PTP1B using siRNA also reduced Src kinase activity and colony formation ability of colon cancer cells. PTP1B siRNA reduced tumor growth in nonobese diabetic/severe combined immunodeficient mice. This study suggests that (a) PTP1B can act as an important activator of Src in colon cancer cells via dephosphorylation at Y530 of Src and (b) elevated levels of PTP1B can increase tumorigenicity of colon cancer cells by activating Src. [Cancer Res 2007;67(21):10129–37]

Introduction

Cancer of the colon and rectum is the second leading cause of cancer death in North America. Colon carcinogenesis has been believed to be a multistep process involving gene mutations in APC, K-ras, and p53 (reviewed in ref. 1), but the recent discovery of heterogeneous patterns of tumor mutations suggests that multiple alternative genetic pathways and/or other mechanisms might also lead to the development of colorectal cancer (2). Src kinase activity is elevated in a high proportion of colon human carcinoma cell lines and tissues (3–5), and this elevation has been shown to contribute to the tumorigenicity of colon cancer cells (6, 7). Src specific activity has been examined in several cell lines and tissues and has been shown to contribute most to the elevation in the Src kinase activity in most of the cell lines and tissues (3–5). In a subset of advanced human colon cancers, this elevation of Src specific activity (activation of Src) has been linked to a truncating mutation in Src in the codon of amino acid 531 (8). However, in most colon cancers, the cause is unknown.

Src kinase activity can be regulated in several ways. Two major phosphorylation sites are present on human Src: the autophosphorylation site Y419 and the negative regulatory COOH-terminal phosphorylation site Y530 (reviewed in ref. 9). Phosphorylation at Y419 allows Src increased access to substrates to facilitate Src activation (10). Phosphorylated Y530 interacts with the SH2 domain of Src, resulting in the suppression of its kinase activity (11). Y530 of Src is phosphorylated by Csk, a ubiquitously expressed protein kinase (12). In addition, Csk homologous kinase has been shown to perform a similar role in brain and hematopoietic cells (13). Several protein tyrosine phosphatases are capable of activating Src by dephosphorylating Y530 (reviewed in ref. 9). These include PTP-α, PTP-λ, SHP-1, SHP-2, and PTP1B. Among them, PTP-α has been shown to activate Src during mitosis (14) and in neuronal differentiation (15, 16). PTP-λ has been implicated in regulating Src in osteoclast precursor cells (17). PTP1B has been shown to activate Src in focal adhesions and integrin signaling (18–20) and in insulin signaling (21) and has been identified as the major tyrosine phosphatase activity capable of dephosphorylating FCP in a breast cancer cell line MDA-MB-435S (22). (FCP consists of human Fyn residues 503 to 537, a region that is conserved among various Src family members. Dephosphorylation of this peptide can be used to evaluate PTase activity that dephosphorylates Src at Y530). Phosphorylation at other sites in Src may also be involved in partially regulating Src kinase activity. These include phosphorylation of Src (chicken) at T34, T46, and S72 during mitosis (23) as well as Y138 (mouse Src; ref. 24) and Y213 (chicken Src; ref. 25) in platelet-derived growth factor (PDGF) signaling. Besides phosphorylation-mediated regulation, Src activity can be regulated through interaction with many receptors and adaptor proteins (reviewed in ref. 9).

In this study, we used well-established human colon cell lines as a model system to study the mechanism of Src activation in human colon cancer. We report herein that there was a consistent pattern between the cancer cell lines and a normal colon cell line examined, suggesting that dephosphorylation of the negative regulatory Y530 phosphorylation site of Src by PTP1B is a common mechanism of Src activation in a high percentage of human colon cancer cell lines. We also showed the novel oncopgenic property of PTP1B in colon cancer cells.

Materials and Methods

Materials. Full-length wild-type human PTP1B in the pFLAG-CMV-2 mammalian expression vector was constructed as described (22) using the p3Hl vector containing full-length human PTP1B, a kind gift of Dr. Jonathan Chernoff (Fox Chase Cancer Center, Philadelphia, PA). Small interfering RNA (siRNA) duplexes of human Src (sense, 5′-UCCGAGCCUCUCAACUCCUUdTdT-3′; antisense, 5′-AGGAGUUGAGCCUCCGAdTdT-3′) and human PTP1B
(sense, 5′-UAGGUACAGACGCUAGUdTdT-3′; antisense, 5′-ACUGACGUUCUCGUAUdTdT-3′) were synthesized at the University of Calgary synthesis facility. Negative control siRNA (sense, 5′-UUCUCCGAACGUUGACACGUdTdT-3′; antisense, 5′-ACUGACGUUCUCGAGAAdTdT-3′) was from Qiagen. CinnGEL 2Me (cell-permeable PTPIB inhibitor) was from BIOMOL. Polyclonal and monoclonal anti-PTPIB (Ab-1) antibodies were from Upstate and Calbiochem, respectively. Anti-Src monoclonal antibodies MAE2-17 and MAE327 were from Quality Biotechnology and from a hybridoma provided by Dr. Joan Brugge (Harvard Medical School, Boston, MA), respectively. Polyclonal phosphorylated Src family (Y419) antibody (anti-Y419-P) and phosphorylated Src (Y530) antibody (anti-Y530-P) were from Cell Signaling. Monoclonal anti-tubulin (Ab-1) was from Oncogene Sciences. Polyclonal calnexin (H-70), Csk (C-20), Src2, and normal monoclonal IgG were from Santa Cruz Biotechnology. Monoclonal anti-PTP-α was from BD Transduction Laboratories. Polyclonal anti-phosphorylated insulin receptor antibody (IR-Y1162/1163-P) was from Upstate.

**Cell lines and culture.** SW48, DLD-1, HCT 116, and HT-29 [from the American Type Culture Collection (ATCC)] were grown in DMEM supplemented with 10% fetal bovine serum (FBS), COLO 201 (ATCC) was grown in DMEM supplemented with 10% FBS and 1 mmol/L sodium pyruvate. Caco-2, LS 174T, and CCD-18Co (ATCC) were grown in MEM supplemented with 10% FBS and 1 mmol/L sodium pyruvate. FHC (ATCC) was cultured in the complete growth medium according to ATCC.

**Preparation of cell lysates.** Subconfluent cells in tissue culture dishes were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer freshly supplemented with phosphatase and protease inhibitors (22). After 20 passes through a 21-gauge needle, lysis buffers were clarified by centrifugation at 10,000 × g for 20 min. Protein was quantified using the Bradford assay. Tumor tissues were first homogenized in RIPA lysis buffer using a Dounce homogenizer with 50 strokes followed by same procedures starting from needle passes.

**Western blotting and protein quantification.** Proteins were separated on SDS-PAGE gels followed by transfer to polyvinylidene fluoride membranes. Blots were incubated with the appropriate primary antibody in TBS-Tween 20 at room temperature for 1 h. Following incubation with an anti-mouse or anti-rabbit secondary antibody conjugated to horseradish peroxidase, the blots were visualized using enhanced chemiluminescence (ECL) Plus from Amersham. Quantification of bands was done using ImageQuant software on a Storm 860 PhosphorImager from Molecular Dynamics unless stated otherwise.

**Immunodepletion.** Anti-PTPIB (Ab-1), anti-PTP-α, or control mouse IgG antibody was preimmobilized on protein G-agarose by incubation for 1 h at 4°C on a rotator. The antibody beads were then used to deplete PTPIB or PTP-α with detailed procedures as described previously (22).

**Membrane preparation.** Total cell membrane fractions were prepared as described (26).

**Labeling of phosphatase substrate and phosphatase assay.** 32P-labeling of FCP by Csk was described previously (22). Phosphatase assay reactions contained 10 μmol/L phosphorylated FCP peptide and 0.3 μg membrane fraction in a final volume of 200 μL dephosphorylation buffer as described previously (22). These were incubated at 30°C for up to 10 min during which time 30 μL aliquots were removed at 2-min intervals. Reactions were stopped by addition of 50 μL 20% trichloroacetic acid. Inorganic 32P was removed and counted in a scintillation counter as described (27).

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**Immunoprecipitation and kinase assay.** Cell lysates were incubated with MAE327 antibody, and the immune complexes were assayed using Src optimal peptide as described previously (22).

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**Transfection.** DLD-1 and SW48 cells were transiently transfected with plasmid DNA (pFLAG-CMV-2-PTPIB, pFLAG-CMV-2, or EGFP C1) using Lipofectamine 2000 (Life Technologies), or siRNA (200 nmol/L, unless stated otherwise) using Oligofectamine (Life Technologies), as per the manufacturer's instructions. Cells were collected 24 h later for soft agar assay, Western blotting (48 h after siRNA transfection), and Src kinase assay (48 h after siRNA transfection). For DNA double transfection, cells were transfected on 2 consecutive days before lyses (48 h after first transfection).

**Monolayer growth.** Equal number of cells (8 × 10^5 for PP2 assays; 10.5 × 10^3 SW48 cells and 7.4 × 10^3 DLD-1 cells for CinnGEL, 2Me assays) were seeded in 3.5-cm dishes at ~5% confluence and grown in medium in the presence or absence of chemical inhibitors (0.5 μmol/L PP2 in 0.1% DMSO or control 0.1% DMSO; 5 μmol/L CinnGEL 2Me in 0.1% DMSO or control 0.1% DMSO) for 4 days before reaching 75% confluence. The medium containing the inhibitors was changed on day 3. The cells were trypsinized after 4 days and counted with a Coulter counter.

**Soft agar colony assay.** Equal number of viable cells (2 × 10^4 for SW48 or DLD-1; 1 × 10^3 for COLO 201) were added to 3 mL DMEM containing 10% FBS, 100 units/mL penicillin G and streptomycin, and 0.3% agarose. After vortexting, the plates were plated on 60-mm dishes over a 3 mL.
bottom layer of prehardened 0.5% agarose medium. In PTP1B and Src inhibition assays, agar contains 0.1% DMSO in the presence or absence of 5 μmol/L CinnGEL 2Me and 0.5 μmol/L PP2. Additional 0.33% agarose medium (in the presence or absence of inhibitor) was added every 3 days. Cells were maintained in 37°C in 5% CO2 incubator for 9 days, and colonies containing >100 cells were counted with an inverted microscope and plates were photographed.

Tumorigenicity assay in mice. SW48 cells were transfected with either PTP1B siRNA or control siRNA. At 24 h after transfection, the trypsinized cells were spun down with low-speed centrifugation and washed twice with and resuspended in a medium containing 50% DMEM without phenol red and 50% PBS. Cells (10^6) in 0.2 mL of this medium were inoculated s.c. on the dorsal side of each 7-week-old male immunodeficient nonobese diabetic (NOD)/severe combined immunodeficient (SCID) mice (four mice in each group). The mice were sacrificed after 4 weeks and the tumors that formed at the site of injection were excised, weighed, and photographed.

Results

Activation of Src in colon cancer cell lines. We first examined Src specific activity of colon cell lines (Fig. 1A). The Src specific activity was increased greatly in all the epithelial cancer cell lines examined, including SW48, DLD-1, HCT 116, HT-29, Caco-2, COLO 201, and LS 174T, relative to the normal colon epithelial FHC cells, ranging from 5.2-fold to 18.7-fold (Fig. 1A). Therefore, Src was activated in all the colon cancer cell lines examined.

Phosphorylation of Src at Y419 and Y530. Because phosphorylation at Y419 and Y530 (the two major sites of phosphorylation in human Src) can enhance and decrease Src activity, respectively, we examined the phosphorylation of these two tyrosine sites. As shown in Fig. 1B, we observed only minor differences in Y419 phosphorylation (Y419-P/Src) among most of the cell types and no correlation between Y419 phosphorylation status and Src specific activity. Overall, Y419-P/Src in the colon cancer cell lines (average of 0.92) was not higher than that in the FHC normal colon cells (1.00). Hence, phosphorylation at Y419 was unlikely to be a general mechanism responsible for activating Src in the colon cancer cell lines tested.

The COOH-terminal tyrosine phosphorylation of Src (Y530-P/Src) in the same colon cancer cell lines was greatly reduced compared with FHC normal colon cells, with most cell lines exhibiting up to 2- to 3-fold reductions. This suggested that enhanced
dephosphorylation at Y530 is an important mechanism contributing to the activation of Src in these colon cancer cell lines. We also assayed Y530 phosphorylation of MAb327-immunoprecipitated Src and obtained similar results (data not shown). Consistent with the hypothesis above was the observation that three of the four cell lines with the highest Src specific activity (LS 174T, HT-29, and Caco-2) also exhibited the three lowest Y530-P/Src values.

Membrane FCP phosphatase in colon cells. The phosphorylation status at Y530 is determined by a balance between phosphorylation and dephosphorylation. Because there was not much change in the Csk protein level among these cells (data not shown), we wanted to examine if variations in phosphatase activity in the cells might explain the reduced Y530 phosphorylation we observed. To measure the phosphatase activity that specifically recognized the Y530 phosphorylation site of Src, we used FCP as substrate. To further ensure specificity regarding the generally low-specificity nature of PTases in vitro, we assayed the membrane-associated FCP phosphatase activity in all the colon cell lines because Src is predominantly associated with plasma and endosomal membranes (28, 29).

Total cell membrane fractions were prepared according to established protocols (26, 30). Membrane FCP phosphatase activity was higher in all the seven cancer cell lines compared with the normal colon cell line (Fig. 2A and B). In each of the cancer cell lines, the high FCP phosphatase activity was associated with a low level of phosphorylation at Y530 and a high specific activity of Src. To identify the major Src-specific phosphatase(s) activity in the cancer cells, we used immunodepletion assays with anti-PTP1B and anti-PTP-α antibodies. Figure 2C (top) shows that, in four of the colon cancer cell lines (SW48, DLD-1, HCT 116, and HT-29) and in the normal FHC cells, PTP1B was the predominant contributor to the membrane FCP phosphatase activity (~70–80%). In Caco-2 and LS 174T cells, the contribution was ~50%. However, the phosphatase activity in COLO 201 cells was predominantly contributed by PTase(s) other than PTP1B, which at this time are unidentified. Furthermore, when PTP1B levels were knocked down by approximately 50% to 80% by siRNA, the membrane FCP phosphatase activity was decreased by approximately 40% to 63% (Fig. 2C, bottom). This confirms that PTP1B is the major FCP phosphatase activity associated with the membrane in these cells (SW48, DLD, and HCT 116 cells were cell lines in which PTP1B levels could be knocked down by >50%).

We also examined the protein level of PTP1B in the total membrane preparations. As shown in Fig. 2D, PTP1B protein levels varied in the colon cancer cell lines (lanes 1–7), but all were higher than that of FHC cells. Overall, increased PTP1B protein levels are consistent with elevated PTP1B phosphatase activities in the colon cancer cell lines, indicating that enhanced membrane PTP1B activity in most cancer cell lines was at least partially due to an up-regulation of membrane PTP1B protein levels. Differences between PTP1B protein levels and PTP1B phosphatase activities in some of the colon cancer cell lines may be due to variations in posttranslational regulation of PTP1B activity, including tyrosine phosphorylation (31) and redox status (32).

Activation of Src by PTP1B overexpression in colon cancer cell lines. To examine if PTP1B was capable of dephosphorylating and activating Src in colon cancer cell lines, we transfected colon cells with a plasmid expressing wild-type PTP1B. Overexpression of PTP1B in DLD-1 further reduced Src Y530 phosphorylation to ~60% of the level in control DLD-1 cells transfected with control plasmid (Fig. 3A, top). Correspondingly, the endogenous Src kinase activity was increased by ~2-fold (Fig. 3A, top). Similar results were obtained in SW48 cells (Fig. 3A, bottom). For the Src kinase assays, comparable amounts of Src were immunoprecipitated from both control- and PTP1B-transfected cells (Fig. 3A). Overexpression of the phosphatase-deficient mutant PTP1B (C215S) did not cause detectable change in either Y530 phosphorylation or Src kinase activity compared with empty vector–transfected control DLD-1 cells (Fig. 3B), indicating phosphatase activity of PTP1B was important for the Src activation, consistent with previous observations using HEK 293 cells (22). Considering that the transfection efficiency of these cells was ~50% (determined by fluorescence-activated cell sorting (FACS) analysis of cells expressing green fluorescent protein (GFP)), following transfection of EGF C1, it is likely that the level of Src activation would be even higher if the transfection had been complete. To evaluate the effectiveness of ectopic PTP1B in dephosphorylating Y530 phosphorylation, we increased the transfection efficiency in DLD-1 cells to ~80% (GFP, FACS analysis) by using two successive rounds of transfection (double transfection), and this led to a more profound reduction of ~75% in Y530 phosphorylation (Fig. 3C).
Effects of PTP1B overexpression on anchorage-independent cell growth. We first used siRNA to target Src in SW48 and DLD-1 cells to determine the effects of altered Src kinase level on the anchorage-independent growth of cells in soft agar, a property that is highly correlated with oncogenicity (33). We found that suppressing Src with siRNA in these cancer cells could inhibit (by 57% to 67%) their soft agar colony-forming ability (Fig. 4A). This result is consistent with and lends further support to the role that Src plays in colon cancer cells as previously shown by others using HT-29 and KM12C cell lines (6, 7). We next wanted to examine if PTP1B, which increases Src activity, could enhance the colony-forming ability of colon epithelial cells. Colony formation of SW48 cells transfected with PTP1B was increased to 270%, relative to SW48 cells transfected with control vector (Fig. 4B and C, left). In DLD-1 cells, colony formation in the presence of overexpressed PTP1B was increased to 180% of the control (Fig. 4B and C, left). These data, along with Fig. 3, indicate that increasing PTP1B phosphatase levels led to a considerable increase in the oncogenic phenotype of colon cancer cells by activating Src. No apparent changes were observed in the colony formation ability of SW48 or DLD-1 cells transfected with PTP1B (C215S) mutant relative to cells transfected with control vector (data not shown).

To confirm that the effect of PTP1B on anchorage-independent growth was mediated by Src, we tested the results of the Src kinase inhibitor PP2. Figure 4C (left) shows that, in both SW48 and DLD-1 cells, addition of PP2 almost completely suppressed the increase of colony formation caused by PTP1B overexpression, indicating that PTP1B is enhancing anchorage-independent growth via Src activation. PP2 did not have any appreciable inhibitory effects on cell growth in monolayer (Fig. 4C, right), indicating the effect of PP2 and PTP1B on colony-forming ability was specific. Src siRNA also suppressed the increase of colony formation caused by PTP1B overexpression (Fig. 4C), further confirming the PP2 results.

Inhibition of Src and anchorage-independent cell growth by down-regulating PTP1B in colon cancer cell lines. To further confirm the effects on oncogenic potential of PTP1B in the colon cancer cells, we inhibited PTP1B activity using the PTP1B inhibitor CinnGEL 2Me, which resulted in reduced colony-forming ability of colon cancer cells SW48 and DLD-1 (Fig. 5A). CinnGEL 2Me did not have any appreciable inhibitory effects on cell growth in monolayer (Fig. 5A), but in the control COLO 201 cells where PTP1B contributes minimally to the membrane FCP phosphatase activity (Fig. 2C). Moreover, consistent with these data, knocking down the PTP1B protein level using PTP1B siRNA caused a substantial decrease in the colony-forming ability of SW48 and DLD-1 (Fig. 5B).

Consistent with the results of colony-forming assays, treatment of SW48 and DLD-1 cells with the PTP1B inhibitor CinnGEL 2Me resulted in increased Src Y530 phosphorylation and decreased Src kinase activity in these cells (Fig. 5C) but not in the control COLO 201 cells where PTP1B contributes minimally to the membrane FCP phosphatase activity (Figs. 2C and 5C). This result and the enhanced autophosphorylation (Y1162/1163-P) of the IR, another substrate of PTP1B (ref. 34, reviewed in ref. 35), also indicated that CinnGEL 2Me was actively reducing PTP1B phosphatase activity inside cells (Fig. 5C). Reducing endogenous PTP1B activity by using

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Figure 4. Enhancement of colony formation in soft agar by PTP1B overexpression in colon cancer cells. A, suppression of colony formation in soft agar by Src siRNA in SW48 and DLD-1 cells. Cells were transfected with either Src siRNA or control siRNA. After 24 h, one set of the cells was trypsinized and seeded in soft agar medium. Right, >100 colonies appeared on each soft agar plate after growth and were counted microscopically and plotted. At 48 h after transfection, another set of the cells was lysed and 10 μg of whole-cell lysates were separated on SDS-PAGE for Western blot analysis with anti-Src and anti-tubulin antibodies. Left, Src protein levels were measured using quantitative scanning of ECL chemiluminescent bands and normalized to tubulin protein levels in each sample. Results were from two experiments and expressed relative to that in control cells transfected with control siRNA. B, colony formation in soft agar of colon cancer cells after transfection with PTP1B. SW48 and DLD-1 cells were transfected with PTP1B or control plasmid. More than 100 colonies appeared on each soft agar plate and typical portions of the microscopic fields are shown. Colony formation numbers in soft agar of three independent experiments were measured and plotted as shown in C. C, left, colony formation in soft agar of PTP1B-transfected colon cancer cells in the presence and absence of PP2 and Src siRNA. Results were expressed relative to that in control cells transfected with control plasmid in the absence of PP2. Src siRNA was transfected immediately after PTP1B transfection (4 h) was finished. n = 3. Right, effect of PP2 on monolayer cell growth. Equal number of cells was grown in medium in the absence (0.1% DMSO) or presence of 0.5 μmol/L PP2 (0.1% DMSO) and cell number was counted after 4 d and expressed relative to cells in the absence of PP2. n = 3.
PTP1B siRNA also led to increased Src Y530 phosphorylation and decreased Src kinase activity in SW48 and DLD-1 cells (Fig. 5D). The changes in Src kinase activity ranged from 20% to 25%. Figure 5E shows that an approximate 25% reduction in Src activity (obtained by modulating the Src siRNA concentration) in colon cancer cells was biologically significant and could lead to an approximately 49% to 57% decrease in soft agar colony formation, close to that caused by knocking down Src by ~50% (Fig. 4A). These data provide support that PTP1B affects colony formation mainly via Src. Cumulative effects of PTP1B knockdown over the course of several possible cell doubling times involved in the colony-forming assays might contribute to the higher level of inhibition observed in these assays.

We also noticed that knockdown of Src by 50% (Fig. 4A) was more potent than knockdown of PTP1B by much more than 50% (Fig. 5B and D) in reducing soft agar colony numbers. This is probably because Src has a more direct role in promoting anchorage-independent colony formation, in contrast to PTP1B that is presumed to work through Src in this situation.

**Effects of PTP1B on tumor formation ability of colon cancer cells.** Lastly, we examined the ability of SW48 colon cancer cells to produce tumors with and without PTP1B knockdown using siRNA. As shown in Fig. 6A, tumors formed rapidly in SCID mice implanted with control scrambled siRNA-transfected SW48 cells. However, the tumors in mice that arose from the implanted PTP1B-knockdown cells were smaller in size and lighter in weight.
than those arising from the control cells. PTP1B protein levels in the tumors of PTP1B-knockdown origin were lower than those from control tumors, suggesting that, even after 4 weeks, PTP1B siRNA was still exerting some effects in the tumor cells (Fig. 6B). A time course of PTP1B levels after transfection of SW48 cells with PTP1B siRNA and growth in cell culture also showed that the effect of PTP1B siRNA in SW48 cells lasted quite long, with an efficient knockdown over 13 days (Fig. 6C).

Discussion

Src specific activity has been reported to be elevated in a high proportion of human colon cancer cell lines and tumors (3, 4), but the magnitude of this elevation was not extensively quantitated. In one subset of advanced human colon cancers, increased Src-specific activity has been linked to a mutation in the COOH-terminal regulatory domain of Src (8). However, in most colon cancers, the cause is unknown. In this article, we report that there was a consistent pattern among the numerous cell lines examined, which suggests a common mechanism that could contribute to Src activation in a high percentage of human colon cancer cell lines compared with a normal colon cell line.

We found that there was a substantial increase in Src-specific activity, ranging from 5.2- to 18.7-fold, in all seven of the human colon cancer cell lines examined relative to the normal human colon cell line FHC. This Src activation (increase in specific activity) may increase its phosphorylation efficiency, which is particularly important when there are a limited number of substrates available to Src in specific subcellular locations or a limited time for Src to phosphorylate substrates at these sites. This agrees with evidence that chicken Src does not induce significant neoplastic transformation when overexpressed in chicken or rodent cells, in contrast to activated forms of Src (36–38). Our data show that differences observed in Src specific activity in both normal and colon cancer cell lines were not highly correlated with phosphorylation at Y419. In contrast, we determined that a considerable reduction in the phosphorylation level of Y530 accompanied the elevation in Src specific activity in all the colon cancer cell lines tested, indicating that this may be a critical feature responsible for Src activation in these colon cancer cell lines.

By measuring phosphatase activity directed against the FCP, we have previously shown up-regulation of an unidentified protein tyrosine phosphatase activity in human melanocytes and in breast cancer cell lines that possessed activated Src and reduced phosphorylation at Src Y530 (26, 39, 40). We identified PTP1B as the major tyrosine phosphatase activity capable of dephosphorylating FCP in a breast (epithelial) cancer cell line MDA-MB-453S (22), suggesting that PTP1B might activate Src in breast cancer cells. In the present report, we determined that PTP1B was the major tyrosine phosphatase activity capable of dephosphorylating FCP in six of seven human epithelial colon cancer cell lines and that phosphatase activities were greatly elevated in these colon cancer cell lines compared with a normal colon epithelial cell line. We further showed that elevated PTP1B activity contributed to Src activation in these colon cancer cells through experiments involving a PTP1B phosphatase inhibitor, siRNA inactivation of PTP1B, and through PTP1B overexpression experiments. In summary, our report suggests a role for PTP1B activation of Src in cancer cells in vivo and also indicated that this seems to be a mechanism occurring in a high percentage of colon cancer cell lines. Previously, in a small subset of advanced colon cancers, Src activation has been attributed to a mutation in Src (8). Our report suggests that PTP1B dephosphorylation of Src Y530 may be another mechanism of Src activation, possibly in a larger subset of colon cancers.

Our results indicated that PTP1B-mediated dephosphorylation of Y530 was largely responsible for the activation and elevation of Src kinase activity observed in most of these colon cancer cell lines. However, this does not exclude other possible mechanisms that might contribute to elevated Src activity in some of these cell lines. For example, potential differences in Csk activity or subcellular localization might also contribute to differences in Src Y530 phosphorylation, although we have not detected a noticeable reduction in Csk protein levels in these cancer cells. Activation of Src may also involve Src-binding proteins that disrupt the intramolecular inhibition of Src without changing its COOH-terminal phosphorylation (reviewed in ref. 9), and Src may also be phosphorylated at other sites (23–25) or have an activating mutation in regions other than in the COOH-terminal regulatory region.

Recently, siRNA knockdown has been shown to be able to last ∼10 days in s.c. tumors in mice and up to 4 weeks in nondividing mouse hepatocytes (41). Our experiments suggested that PTP1B siRNA knockdown could last at least 2 weeks in SW48 cells implanted s.c. in mice. Although the doubling time of SW48 colon cancer cells in tumors is not precisely known, it is ∼1 day in cell culture. Therefore, it is highly likely that effects on proliferation and possibly apoptosis caused by siRNA knockdown over a period of several days to 2 weeks would give rise to the observed difference in tumor size as well as the difference in colony formation in soft agar.
In summary, we have shown that membrane PTP1B phosphatase activity levels were up-regulated in several naturally occurring human epithelial colon cancer cell lines. This could result in Src activation by reducing phosphorylation of Src at Y530, leading to enhanced neoplastic properties, as shown by effects on anchorage-independent growth of SW48 and DLD-1 cells after transfection of PTP1B, and further confirmed by the ability of PTP1B siRNA in reducing tumor growth in NOD/SCID mice. Although whether the effect of PTP1B siRNA on tumor growth was also mediated via the inhibition of Src activation has not been determined, it is highly likely that the mechanism is similar to what we found for anchorage-independent growth, which is highly correlated to tumorigenicity (33). This report is among the first descriptions of the ability of PTP1B to enhance the neoplastic phenotype of cells. Previously, PTP1B has been shown to down-regulate insulin signaling (reviewed in ref. 42) and regulate integrin (18, 19, 43). Interestingly, PTP1B has also been shown to be able to suppress cellular transformation by several oncogenic protein tyrosine kinases, such as v-src, in mouse/rat fibroblasts (46, 47). This may be due to a quite different cellular context between the above genetically manipulated murine fibroblasts and naturally occurring human epithelial colon cancer cells. In fact, PTP1B has been reported to activate Src in 293 cells, L cells, and platelets but not in murine fibroblasts and COS-7 monkey kidney fibroblasts (18, 19, 22, 48, 49). Furthermore, in the case of transformation by v-src in murine fibroblasts, PTP1B could not dephosphorylate and activate v-src, which lacks a negative regulatory tyrosine residue due to its truncation at its COOH terminus, whereas cellular signaling proteins hyperphosphorylated in the presence of v-src could become substrates of overexpressed PTP1B.

Recently, some PTP1B inhibitors have been developed as potential therapeutics in the treatment of type 2 diabetes and obesity based on the observation that PTP1B has been identified as a major negative regulator of both insulin and leptin signaling (reviewed in ref. 50). We have now shown that inhibition of PTP1B can reduce the oncogenic properties of colon cancer cells. The link between oncogenic potential and PTP1B may lead to the use of PTP1B inhibition as a cancer therapeutic in certain types of cancer, such as colon and breast cancer.

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PTP1B Contributes to the Oncogenic Properties of Colon Cancer Cells through Src Activation

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