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Celecoxib inhibits the expression of survivin via the suppression of promoter activity in human colon cancer cells

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ABSTRACT

We investigated the effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on human colon cancer cell lines to clarify the mechanisms underlying the chemopreventive effect of NSAIDs. Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, induced apoptosis and strongly reduced the expression of an anti-apoptotic protein, survivin, in both protein and mRNA levels in HCT-116 cells. Subsequently, we conducted luciferase reporter assay using a reporter gene driven by the human survivin promoter. A series of analyses using luciferase reporter constructs containing fragments of the survivin promoter and electrophoretic mobility shift assay indicated that the −75/−66 bp region relative to the initiating codon was involved in celecoxib action to suppress survivin promoter activity. Celecoxib also suppressed the activity of TOPflash, T-cell factor reporter plasmid, and the reporter gene driven by the human cyclin D1 promoter, suggesting that this compound inhibited the expression of Wnt/β-catenin signaling target genes. Further, we found that other NSAIDs including indomethacin, resveratrol, and SC-560 induced apoptosis and suppressed the expression of survivin and the Wnt/β-catenin signaling pathway in HCT-116 cells, indicating that these effects were likely to be common among NSAIDs. Moreover, NSAIDs (celecoxib, SC-560 and indomethacin) also suppressed the expression of cyclin D1 and survivin on other colon cancer cell lines (DLD-1 and SW-620). Our results suggested that NSAIDs could inhibit proliferation and induce apoptosis in colon cancer cells by inhibition of survivin expression and the Wnt/β-catenin signaling pathway.

1. Introduction

Apoptosis is a morphologically distinct form of genetically regulated cell death and provides a vital protective mechanism against the development of neoplasia by removing cells with DNA damage [1]. Inhibition of apoptosis confers a survival advantage on cells harboring genetic alterations and promotes the acquisition of further mutations to increase neoplastic progression [2]. The majority of cancers, including colon cancer, are known to overexpress anti-apoptotic proteins, such as survivin, to protect cells from apoptosis [3–5]. Levels of survivin expression in tumors have been reported to be correlated with aggressive phenotypes and a poor prognosis [6,7]. In human colorectal tumorigenesis,

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Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; COX-2, cyclooxygenase-2; FBS, fetal bovine serum; TCF, T-cell factor

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survivin has been thought to play an important role in the progression of cellular dysplasia from adenoma to carcinoma [8]. Survivin gene expression has been reported to be upregulated by Akt activation [9] and recent reports indicated that adenomatous polyposis coli (APC) regulates survivin expression in colonic crypts and that survivin is a target gene of the Wnt/β-catenin signaling pathway [10,11]. Most colorectal cancers have somatic mutations in APC or β-catenin, which are members of the Wnt/β-catenin/T-cell factor (TCF) signaling pathway [12–14]. Although this pathway is essential to regulate gene transcription during embryonic development, it is probably present in intestinal crypts throughout adult life, maintaining the balance between cell proliferation and differentiation [14–16].

Numerous experimental and epidemiological studies in human suggest that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have chemopreventive activity against colon cancer [17–19]. Several randomized trials have shown the growth inhibition of polyps and decrease in number of existing polyps in patients with familial adenomatous polyposis (FAP) who received sulindac or celecoxib [20,21]. Two randomized placebo-controlled studies have shown that aspirin reduced the risk of colorectal adenomas among population with previous colorectal cancer and adenoma, excluding FAP patients [22,23]. The possible cellular mechanisms underlying these chemopreventive effects of NSAIDs have been thought to be the induction of apoptosis, cell-cycle arrest and the inhibition of angiogenesis [18,19]. Moreover, NSAIDs have been reported to inhibit the Akt [24,25] and the Wnt/β-catenin signaling pathways [26–29].

Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, is the only NSAID approved by the Food and Drug Administration for the treatment of FAP patients. However, the molecular mechanism responsible for the chemopreventive effect of celecoxib is not entirely understood. In this study, we found that celecoxib induced apoptosis and suppressed the survivin expression in HCT-116 cells. Therefore, we tried to identify the mechanism by which celecoxib reduces survivin expression. Our results suggested that the −75/−66 bp region relative to the initiating codon in the survivin promoter played an important role to mediate celecoxib action. Moreover, we found that not only celecoxib but also other NSAIDs suppressed the expression of cyclin D1 and survivin in HCT-116, DLD-1 and SW-620 cells, suggesting that NSAIDs inhibit cell proliferation and induce apoptosis in colon cancer cell lines.

2. Materials and methods

2.1. Chemicals and antibodies

Wild-type cyclin D1 pGL3 basic luciferase reporter construct and its mutant were generous gifts from Drs. O. Tetsu and F. McCormick, University of California, San Francisco. TOPflash (TCF reporter plasmid), FOPflash (negative control to TOPflash) and the monoclonal anti-histone H3 antibody were purchased from Upstate Biotechnology (Lake Placid, NY). The polyclonal anti-survivin antibody was from R&D Systems (Minneapolis, MN). The monoclonal anti-GAPDH antibody was from Abcam (Cambridge, UK). The polyclonal anti-phospho-Akt (Ser^{473}) and the polyclonal anti-Akt antibody were from Cell Signaling Technology (Danvers, MA). The polyclonal anti-cyclin D1 antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The monoclonal anti-β-catenin antibody was from BD Biosciences (San Jose, CA). The monoclonal anti-β-actin antibody was from Sigma (St. Louis, MO). Indomethacin was from Wako Pure Chemical Industries (Osaka, Japan). Resveratrol and SC-560 were from Cayman Chemical (Ann Arbor, MI). Celecoxib was kindly provided by Pfizer.

2.2. Cell culture

HCT-116 cells, human colon cancer cell line expressing wild-type APC and mutated β-catenin [12], and DLD-1 and SW-620 cells, human colon cancer cell lines expressing mutated APC and wild-type β-catenin [13,30], were grown in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin G, and 0.1 μg/ml of streptomycin.

2.3. DNA electrophoresis

To detect DNA ladder formation, DNA was extracted from both detached and adherent cells, and was subjected to electrophoresis using 2.0% agarose gel as described previously [31]. An optical densitometric scan of the band in ladder was performed using Science Lab 99 Image Gauge software (Fuji Photo Film) to quantify the DNA ladders.

2.4. Caspase-3 activity assay

Caspase-3 activity was assayed using a cysteine protease protein 32/caspase-3 colorimetric protease assay kit (Medical and Biological Laboratories, Nagoya, Japan). Absorbance at 405 nm was measured to determine caspase-3 activity.

2.5. Purification of nucleic protein

Nucleic protein was purified from cells cultured in 100-mm dish using NE-PER™ nuclear and cytoplasmic extraction reagents (Pierce, Rockford, IL) and subjected to Western blot analysis and electrophoretic mobility shift assay.

2.6. Immunoblotting

Immunoblotting analysis was performed as described previously [32]. Briefly, samples (10 μg/lane) were separated by 15% (for survivin), 12% (for cyclin D1 and Akt) or 10% (for β-catenin) SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoreactive proteins on the membrane were visualized by treatment with a detection reagent (LumiGLO, Cell Signaling Technology). An optical densitometric scan was performed using Science Lab 99 Image Gauge Software.

2.7. Northern blotting

Total cellular RNA was extracted with TRIzol® Reagent (Invitrogen, Carlsbad, CA). The expression of survivin was analyzed by Northern blotting as described previously [31]. Ten micrograms of RNA was used for each lane and