Supplemental Material and Methods

Human Colonic Specimens

Human colonic biopsy specimens were obtained from patients undergoing diagnostic or surveillance colonoscopy for known or suspected UC (receiving either ≥ 2.4 grams 5-ASA/day alone or no therapy) and collected from Northwestern University's Department of Pathology. For comparison, biopsies were obtained from healthy patients undergoing routine colon cancer surveillance. Collection of all patient materials for this study was approved by Northwestern University's Office for the Protection of Human Subjects.

Real Time PCR analysis

Semi-quantitative Real-time PCR analysis was performed using Power Sybr green 2x master mix (ABI) in an ABI 7500 Real Time PCR System on mRNA isolated either from whole biopsy tissue or from epithelial and stromal fractions after laser capture microdissection (LCM). Primers were designed to span genomic DNA intron junctions for specific amplification of mRNA. cDNA from patient biopsies were subjected to 40 amplification cycles and normalized using Gapdh. Fold increases were calculated using the ddCT method normalizing to the average dCT of at least 10 control biopsies.

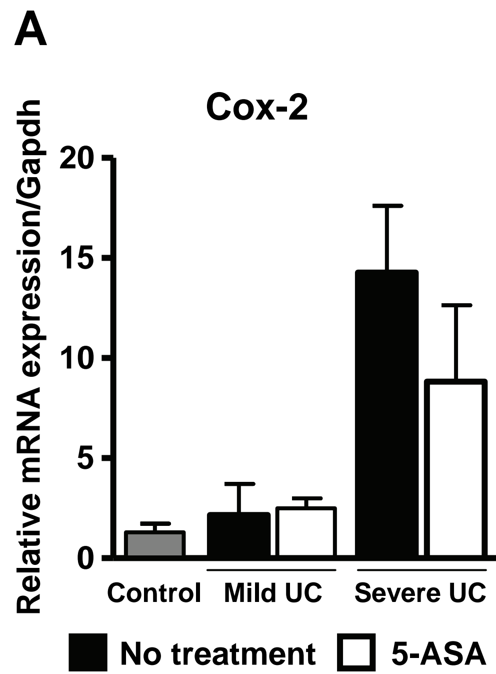
Immunohistochemical Analysis

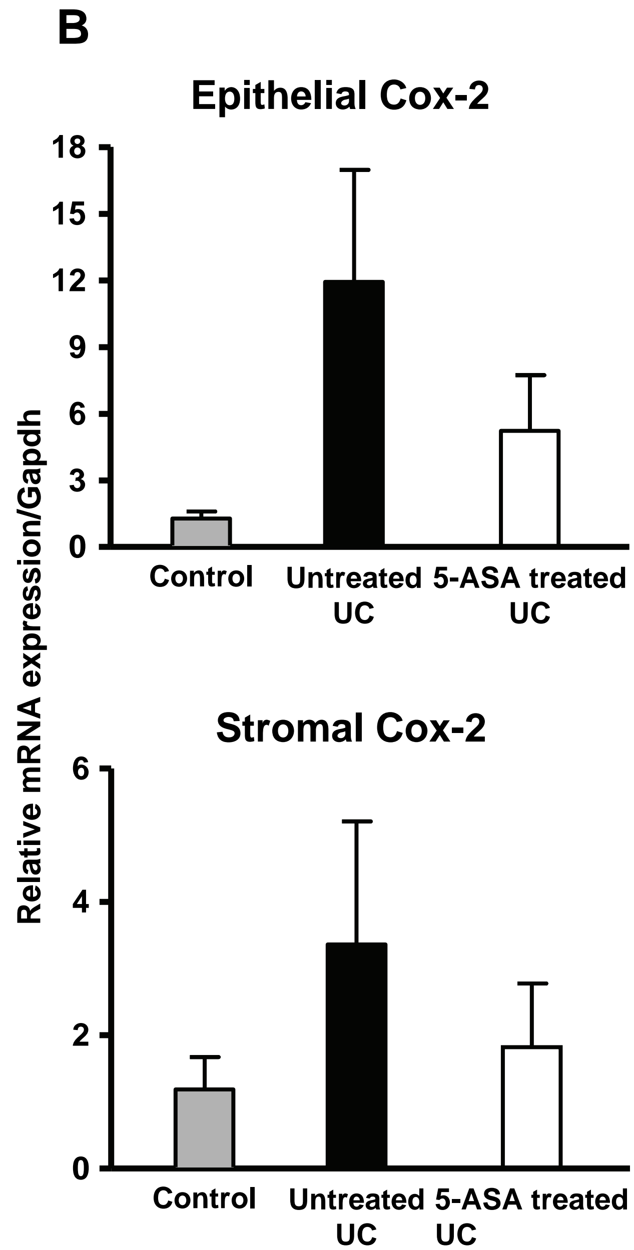
For Bromodeoxyuridine (BrdU; Sigma) labeling, mice were injected with 1mg of BrdU intraperitoneally 2 hours prior to sacrifice. Slides were deparaffinized by incubation in xylene and graded ethanol. Antigen retrieval of formalin fixed paraffin sections was performed using Target retrieval solution (Dako, Carpinteria, CA) within a microwave

(BrdU, P-Akt) or decloaking chamber (P-β-catenin) (Biocare medical, Concord CA). Sections were stained with anti-BrdU Ab (1:250, Accurate Chemical, Westbury, NY), anti-P-Akt T308 Ab (1:100 dilution, Cell Signaling Technology, Davers, MA), or anti-P-β-catenin-Ser552 Ab (1:1,000 dilution, gift from Linheng Li). After overnight incubation, sections were incubated with anti-rabbit or mouse HRP-labeled polymer (Dako) or anti-rat VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA). 3,3'-diaminobenzidine tetrahydrochloride was used to visualize stained cells and sections were counterstained with hematoxylin.

Western Blotting

Proteins were separated by SDS-PAGE, transferred onto a PVDF membrane, blocked with Protein-Free T20 Blocking Buffer (Pierce Scientific, Rockford, IL), and incubated overnight with primary antibody specific for P-β-catenin-Ser 552 or P-Akt S473 (Cell Signaling) followed anti-rabbit secondary antibody. Bands on the autoradiograms were quantified by densitometry using Eagle Eye analyzer (Strategene, La Jolla, CA). The immunoreactivity of each protein was normalized to β-actin and fold-induction was determined relative to non-colitic mice.


A
Cox-2
Relative mRNA expression/Gapdh
20
15
10
5
0
Control
Mild UC
Severe UC
No treatment
5-ASA


B
Epithelial Cox-2
Relative mRNA expression/Gapdh
18
15
12
9
6
3
0
Control
Untreated UC
5-ASA treated UC
Stromal Cox-2
6
4
2
0
Control
Untreated UC
5-ASA treated UC