Supporting Materials and Methods

PCR-Based Subtractive Hybridization

Subtractive hybridization was performed with the PCR-Select Subtraction Kit (Clontech), according to the manufacturer's protocol. In brief, the mRNA samples from tumor-bearing intestines and normal intestines were reverse-transcribed into two cDNA pools by avian myeloblastosis virus reverse transcriptase, with poly(dT) as the primer. For convenient manipulation, the cDNAs from the two pools were digested with the restriction enzyme *Rsa*I to generate shorter fragments. To screen for clones that had elevated expression in the tumor samples, we ligated different 40-bp DNA adaptors to two separate fractions of the cDNA pool from the tumor samples. These two fractions with adaptors were then annealed with a 3-fold excess of cDNA from the pool of normal tissue. Using primers complementary to the two adaptors, PCR was then performed to amplify the cDNA clones that had not hybridized to the driver cDNA from the normal tissue. In parallel, a similar protocol was also performed to amplify the cDNA clones from normal tissue not hybridized to tumor cDNA. The products of the major steps in this approach, including reverse transcription reaction, restriction digestion, ligation of adaptors, subtraction, and PCR amplification, were each examined by agarose gel electrophoresis.

Differential Screening

The PCR-Select Differential Screening Kit (CLONTECH) was used and the corresponding protocol was followed. In brief, the PCR-amplified cDNA clones described above were subcloned into pGEM-T Easy Vector (Promega), and transformed into XL-2 competent cells (Stratagene). The transformed cells were cultured on LB-agar plates containing ampicillin, 5-bromo-4-chloro-3-indolyl β-D-galactoside, and isopropyl β-d-thiogalactoside, allowing blue-white colony selection. The white colonies were randomly selected and cultured in LB-ampicillin media in 96-well cell culture plates for >3 h. PCR using primers complementary to the two adaptors was performed with cultured cells. The PCR products were individually blotted onto nylon membranes. Four identical blots were generated and hybridized respectively with four [^{32}P]dCTP-labeled cDNA probes: subtracted tumor cDNA (I), subtracted normal tissue cDNA (II), unsubtracted tumor cDNA (III), and unsubtracted normal tissue cDNA (IV). The cDNA clones showing strong signals with probes I and III, but not with probes II and IV, were selected and sequenced on an Applied Biosystems 377 sequencer. The generated sequences were compared with Genbank by using the BLAST search tool.

Nonradioactive *in Situ* Hybridization

The protocol for nonradioactive *in situ* hybridization (1), generously communicated by Chris Iacobuzio-Donahue (The Johns Hopkins University, Baltimore), was followed with minor modifications. The cDNA templates for sense and antisense probes were generated by PCR with gene-specific primers linked to a T7 promoter. The cRNA probes were synthesized by T7 polymerase by using digoxigenin (DIG) RNA labeling mix (Roche Diagnostics, Indianapolis), followed by digestion by RNase-free DNase (Roche). The RNase-free sections (10 μm) were deparaffined and rehydrated, followed by 95° C incubation in citrate buffer (for 30 min),

proteinase K digestion (5 μg/ml in TBS, for 5 min), hydrogen peroxide treatment (3% in TBS, for 15 min); and acetylation by acetic anhydride (0.25% in 0.1 M triethanolamine buffer, for 15 min). The DIG-labeled riboprobe, either antisense or sense, was used at a final concentration of 100–500 ng/ml in the hybridization solution (DAKO). The probes were then pipetted onto the sections covered by HybriWell (Sigma) chambers. The hybridization systems were incubated at 65° C for 15 min to denature the probes and hybridized at 42–44° C overnight. The chambers were then removed and the sections were digested with RNase A (25 μg/ml) at 37° C for 30 min, followed by stringent washes (twice for 20 min at 48–52° C in 1x SSC, 50% formamide and 20 min at 57–60° C in 0.1× SSC Stringent Wash from DAKO). The sections were incubated with blocking buffer and then with rabbit anti-DIG IgG for 30 min each. The signals were amplified by incubation with biotinyl-tyramide and strepavidin-horseradish peroxidase from the DAKO GenePoint kit. The chromogenic reactions were performed with 3,3'-diaminobenzidine (DAB) provided in the GenePoint kit. The slides were then counterstained by hematoxylin for 20 sec.

Immunohistochemistry

Immunohistochemistry was performed as described (2). The sections (10 μm) were deparaffined in xylene, rehydrated in ethanol (100%, 90%, 70%, 0%), antigen-retrieved by microwaving, and treated with 3% hydrogen peroxide to quench endogenous peroxidase activity. Normal goat serum was used to reduce nonspecific binding. Sections were then incubated overnight at 4° C with or without the anti-clusterin antibody (a generous gift from Michael Griswold, Washington State University, Pullman, WA). Slides were washed three times (10 min each) with PBS. The biotinylated secondary antibody (goat anti-rabbit, 1:200 dilution) was then applied on the sections for 1 h. After three washes (10 min each) with PBST (PBS with 0.1% Tween 20), the sections were incubated with avidin-horseradish peroxidase (ABC kit, Vector Laboratories) for 1 h followed by another PBST wash (three times for 10 min). The chromogenic reaction was performed with DAB (Sigma). The slides were finally counterstained with hematoxylin for 20 sec.

Immunohistofluorescence Assay

The initial steps for the immunohistofluorescence assay were the same as those for immunohistochemistry, without hydrogen peroxide treatment. The secondary antibody was replaced by FITC-labeled goat anti-rabbit antibody or rabbit anti-mouse antibody conjugated with rhodamine and incubated for 1 h in the dark. After PBST washes (three times for 10 min), the sections were counterstained with 4',6-diamidino-2-phenylindole (1 μg/ml; Sigma) and covered by coverslips without dehydration.

1. Iacobuzio-Donahue, C. A., Argani, P., Hempen, P. M., Jones, J. & Kern, S. E. (2002) *Cancer Res.* **62**, 5351–5357.

2. Cormier, R. T., Hong, K. H., Halberg, R. B., Hawkins, T. L., Richardson, P., Mulherkar, R., Dove, W. F. & Lander, E. S. (1997) *Nat. Genet.* **17**, 88–91.