



The APC gene product in normal and tumor cells

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ABSTRACT The APC gene has been found to be mutated during the development of sporadic colorectal tumors as well as in the germ line of familial adenomatous polyposis patients. To facilitate the characterization of both normal and mutant APC protein, a series of monoclonal and polyclonal antibodies specific for the APC protein was produced. When lymphoblastoid cell lines derived from seven familial adenomatous polyposis patients with known mutations were analyzed by Western blot, an ≈300-kDa protein corresponding to the predicted size of full-length APC was detected in all 7 cell lines. In addition, truncated APC proteins corresponding to the product of the known mutated alleles could be detected in 4 of the 7 lines. Similar analysis of 23 colon carcinoma and 9 adenoma cell lines revealed truncated proteins in 24 (75%) of the cell lines. Moreover, 26 (81%) of the colon tumor lines were totally devoid of the normal, full-length protein. In contrast, Western blot analysis of 40 cell lines derived from sporadic tumors of other organs detected only full-length APC. Immunohistochemical analysis of APC in normal colonic mucosa revealed cytoplasmic staining with more intense staining in the basolateral margins of the epithelial cell. This staining was markedly increased in the upper portions of the crypts, suggesting an increased level of expression with maturation. These studies provide some initial clues to the function of the cytoplasmic protein APC and demonstrate the feasibility of identifying APC mutations by direct analysis of the APC protein.

Familial adenomatous polyposis (FAP) is an autosomal dominant disease in which affected individuals develop hundreds to thousands of benign colorectal tumors (adenomas). Some of these tumors, if not removed, invariably progress to malignancy (carcinomas). Recently, a candidate tumor suppressor gene from chromosome 5q21, APC, was isolated and implicated in the development of FAP (1–4). Further analyses of the APC gene in 150 kindreds indicate that APC mutations can account for most if not all cases of FAP (5, 6). Carcinomas in FAP patients, however, account for <1% of all colorectal cancers. Most colorectal cancers do not have a well-recognized inherited basis and therefore are classified as sporadic. Recent studies have indicated that the majority of these sporadic tumors have somatic mutations of the APC gene (7, 8). Furthermore, these mutations appear to occur early during colorectal tumorigenesis and have been detected in tumors as small as 0.5 cm in diameter (8). The nature of the mutations identified in sporadic tumors and in FAP patients is striking, with 94% of the mutations predicted to result in truncation of the APC gene product. Taken together, the above studies suggest that APC plays an important and early

role in the development of the major forms of colorectal neoplasia.

The APC gene contains an 8538-bp open reading frame and is predicted to encode a 2843-aa polypeptide with few homologies to other proteins (1, 3). The large size of the APC coding region makes identification of mutations labor intensive and costly. However, because the vast majority of mutations identified to date are predicted to truncate the APC protein, many mutations might be more conveniently detected through analysis of the APC protein. To evaluate this approach and to begin investigation of the biologic properties of APC, monoclonal and polyclonal antibodies were produced and used to characterize APC protein in normal and tumor cells.

MATERIALS AND METHODS

Cell Lines. The SW480 cell line is a colorectal cancer cell line (9) in which the status of APC has previously been reported (2). The FAP colon adenoma (BH, FF, and AA), the sporadic colon adenoma (AN, BR, RG, RR, WG, and Vaco 235), the FAP colon carcinoma (JW), and the sporadic colon carcinoma (CMA, KS, Vaco 241, Vaco 206, Vaco 394, Vaco 264, Vaco 478, Vaco 441, Vaco 489, Vaco 425, Vaco 486, Vaco 481, Vaco 451, Vaco 456, Vaco 9M, Vaco 5, Vaco 6, Vaco 8, Vaco 410, Vaco 429, Vaco 444, and Vaco 503) cell lines were established as described (10–14). Most of the breast cancer (MCF7, MDA-MB-231, MDA-MB-468, Hs 578T, T-47D, ZR-75-1, and MDA-MB-435), prostatic cancer (LNCaP, DU 145, PC-3, PPC-1, Du Pro, and Tsu-Prl), cervical cancer (ME-180, MS751, C33a, HeLa, Caski, HT-3, SiHa, CH II, and 8217), pancreatic cancer (MIA PaCa-2, Hs 766T, Capan-1, Capan-2, AsPC-1, BxPC-3, PANC-1, Su86-86, and CFPAC-1), and lung cancer (A427, Calu-1, Calu-6, A-549, A2182, SW1272, Hut292-Dm, 866MT, and DMS92) cell lines are available from the American Type Culture Collection.

Tissues. Colonic mucosa from control and FAP individuals were obtained from surgical resections and frozen immediately. Peripheral mononuclear cells were isolated from EDTA anticoagulated blood by using a Histopaque 1077 step gradient (Sigma) according to the manufacturer's protocol.

Construction of Bacterial Fusion Proteins. A bacterial fusion protein containing the amino-terminal portion of the APC protein was constructed from partial APC cDNA clones as follows. A fragment of APC containing nucleotides –15 to 734 was engineered to have an EcoRI site 10 nucleotides 5' of the initiating methionine by PCR amplification with the following primers: 5'-CAAGGGAATTCAAGGATG-3' and

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Abbreviations: FAP, familial adenomatous polyposis; GST, glutathione S-transferase.

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5'-TGCTTCTGTTGCTTGAGGC-3' (underlined bases were altered to create the *EcoRI* site). After digestion with *EcoRI*, a fragment extending from the engineered *EcoRI* site to the endogenous *EcoRI* site at nucleotide 660 was cloned into the *EcoRI* site of pATH3 (15) and pGEX-3X (Pharmacia), resulting in a trpE fusion (pATH-NAPC) or a glutathione S-transferase (GST) fusion (pGEX-NAPC). A second nonoverlapping fusion protein was constructed from an APC *EcoRI* fragment extending from the endogenous *EcoRI* site at nucleotide 660 to an exogenous *EcoRI* site at 3309 introduced during cDNA cloning. This fragment was cloned into pATH3 and pGEX-2T (Pharmacia) resulting in a trpE fusion (pATH-MAPC) or a GST fusion (pGEX-MAPC). The pATH-encoded trpE fusion proteins (trpE-NAPC and trpE-MAPC) were produced in temperature-sensitive *Escherichia coli* strain CAG456 (16). Stationary-phase *E. coli* were diluted 1:5 and induced for 12 hr by tryptophan starvation. The cells were harvested by centrifugation and resuspended in 1/60th volume of TEN (50 mM Tris-HCl, pH 8/1 mM EDTA/50 mM NaCl). Lysozyme was added to 20 mg/ml, and the culture was incubated on ice for 15 min. After addition of Nonidet P-40 to a final concentration of 0.25%, the sample was sonicated, and the insoluble fusion proteins were pelleted ($12,000 \times g$ for 5 min at 4°C), washed with TEN, and frozen at -80°C. The pGEX-encoded GST fusion proteins (GST-NAPC and GST-MAPC) were produced in *E. coli* JM101 cells. Cells were grown at 37°C to an optical density of 0.5 at 550 nm and induced by the addition of isopropyl β -D-thiogalactoside (0.5 mM). After a 4-hr induction, the cells were harvested by centrifugation, resuspended in 1/30th volume of MTPBS (150 mM sodium chloride, 16 mM monobasic sodium phosphate/4 mM dibasic sodium phosphate, pH 7.3), and sonicated. The insoluble fusion proteins were pelleted, washed once with 0.03% SDS, and frozen at -80°C.

Polyclonal Antibody Production. The insoluble bacterial pellets containing the trpE bacterial fusion proteins were purified by SDS/PAGE. Gel slices containing $\approx 300 \mu\text{g}$ of fusion protein were homogenized in Freund's complete adjuvant (Sigma) for the primary injection and Freund's incomplete adjuvant (Sigma) for booster injections. Two New Zealand White rabbits were injected with each protein preparation. Rabbits were injected every 2 weeks and bled 10 days after each booster injection. The antibodies were affinity purified by binding to the insoluble GST bacterial fusion proteins. The bacterial pellets containing 200 μg of fusion protein were thawed, washed with TEN, and pelleted. One milliliter of antiserum was added to the pellet and incubated on ice for 1 hr. The antibody-insoluble protein complexes were then pelleted and washed with Tris-buffered saline (TBS; 0.1 M Tris-HCl, pH 7.5/0.9% NaCl). The antibodies were released with 500 μl of 0.2 M glycine (pH 2.3) on ice for 5 min. The remaining insoluble proteins were pelleted. The antibody solution was neutralized with 70 μl of 1 M Tris (pH 9.5). Bovine serum albumin and sodium azide were added to a final concentration of 5 mg/ml and 0.05%, respectively.

Monoclonal Antibody Production. Female CB6 F1 mice (Charles River Breeding Laboratories) were immunized by intraperitoneal injection on three successive occasions with 20 μg of trpE-NAPC fusion protein that had been expressed in *E. coli* and purified by electroelution following SDS/PAGE. Hybridomas were produced as described (17), except fused cells were plated at a density of $1 \times 10^6/\text{ml}$. Test bleeds and hybridomas were screened for anti-APC reactivity by ELISA using purified GST-NAPC (18) coated on plates at a concentration of 500 ng/ml. Purified GST-MDM2 was used as the negative control antigen (19). Antigen-antibody complexes were detected by incubation with horseradish peroxidase-conjugated goat anti-mouse IgG heavy and light chain (Kirkegaard and Perry Laboratories, Gaithersburg, MD)

followed by development with tetramethylbenzidine as described (17). All positive hybridomas were subcloned twice by limiting dilution. Monoclonal antibodies were initially tested for specific recognition of APC by Western blot using GST-NAPC and trpE-NAPC, with GST-MDM2 and trpE-MAPC as negative controls.

Western Blot Analysis. To facilitate transfer of high molecular weight proteins, electrophoresis was performed in a 3% low melting point agarose gel formed in 0.1% SDS in Tris/borate buffer (89 mM Tris-HCl/89 mM boric acid/2 mM EDTA). Tris/glycine buffer (25 mM Tris-HCl/191 mM glycine/0.05% SDS) was used as the running buffer. Cell protein lysates were prepared by boiling for 5 min in SDS/PAGE loading buffer (63 mM Tris-HCl, pH 6.8/10% glycerol/5% 2-mercaptoethanol/2% SDS/0.025% bromophenol blue). Protein concentrations were determined following amido black staining as described (20), and 100 μg of total protein was loaded in each lane. The proteins were transferred by capillary action to a polyvinylidene difluoride membrane (Immobilon-P; Millipore) in TBS with 0.04% SDS overnight. The filters were briefly rinsed with TBS and blocked with 10% nonfat dried milk/10% goat serum/0.1% Tween 20 in TBS for 1 hr. After incubation with the primary antibody at 1-2 $\mu\text{g}/\text{ml}$ in 5% nonfat dried milk/0.1% Tween 20 in TBS for 2 hr, the filters were washed for 30 min in 0.1% Tween 20 in TBS with three changes. Horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse antibodies (0.05 $\mu\text{g}/\text{ml}$ in 5% nonfat dried milk/0.1% Tween 20 in TBS) were incubated with the filter for 45 min. The filters were then washed six times, 20 min each, with 0.1% Tween 20 in TBS. Peroxidase activity was detected using Amersham's enhanced chemiluminescence detection kit following the manufacturer's protocol.

Cell Fractionation. The procedure was performed as described (21) with the following modifications. Cells were harvested by centrifugation at $200 \times g$ and washed three times with Dulbecco's phosphate-buffered saline (PBS) containing 0.5 mM calcium chloride. The pellet was resuspended in lysis buffer [5 mM Tris-HCl, pH 7.8/2 mM MgCl_2 /1 mM phenylmethylsulfonyl fluoride/leupeptin (Sigma) at 0.5 $\mu\text{g}/\text{ml}$ /trypsin inhibitor (Sigma) at 10 $\mu\text{g}/\text{ml}$]. The sample was then centrifuged at $500 \times g$ to pellet nuclei and unlysed cells. The pellet was washed once with lysis buffer and saved as the "nuclear fraction." The supernatant was centrifuged at $100,000 \times g$ for 1 hr. The resulting pellet was designated the "membrane fraction," and the supernatant was designated the "cytosolic fraction." All three fractions were solubilized directly in SDS/PAGE loading buffer prior to Western blot analysis. These fractions were verified by finding that p53 and DCC-encoded protein were predominantly found in the nuclear and membrane fractions, respectively. (K.J.S. and L. Hedrick, unpublished results).

Immunohistochemical Analysis. Frozen normal colonic mucosa samples were embedded in O.C.T. (Miles) and cut into 12- μm sections. The sections were immediately fixed in 0.3% hydrogen peroxide in absolute methanol at room temperature for 30 min. The tissues were washed three times in PBS. The APC antigen was unmasked using an antigen retrieval system (Biogenex) following the manufacturer's protocol. The tissues were briefly washed three times with H_2O and three times with PBS, blocked with goat serum for 30 min, and then incubated with affinity-purified polyclonal anti-NAPC (1.5 $\mu\text{g}/\text{ml}$) or anti-MAPC antibodies (1.5 $\mu\text{g}/\text{ml}$) or with normal rabbit immunoglobulins (4.5 $\mu\text{g}/\text{ml}$) diluted in goat serum for 2 hr. The tissues were washed with PBS three times for 10 min each. Biotinylated goat anti-rabbit antibody (Vector Laboratories) was diluted 1:200 in goat serum and exposed to the tissues for 30 min. The tissues were washed in PBS three times for 10 min each. Immunoperoxidase staining was performed using the Vectastain elite ABC system (Vector Laboratories) following the manufacturer's protocol. A nu-

clear counterstain was achieved by overlaying the immunoperoxidase-stained tissue with 4',6-diamidino-2-phenylindole at 0.1 mg/ml and viewing under UV excitation. Competition studies were performed using soluble GST fusion proteins purified as described (18). GST-NAPC and GST-MDM2 fusion proteins were included in the primary antibody incubations at final concentrations of 3 μ g/ml and 9 μ g/ml, respectively.

RESULTS

Detection of APC Protein. Bacterial fusion proteins containing various nonoverlapping portions of the APC protein were used to produce a series of monoclonal and affinity-purified polyclonal antibodies. Affinity-purified polyclonal antibodies were produced against either amino acids 1–220 (anti-NAPC) or 220–1103 (anti-MAPC) of APC. Monoclonal antibodies were developed against APC amino acids 1–220. Ten hybridomas were isolated that reacted specifically with APC protein produced in bacteria. Four of these (CC1, CF11, EF4, and FE9) were chosen for further characterization.

The polyclonal and monoclonal antibodies were first used for Western blot analysis of protein lysates from the colorectal cancer cell line SW480 and from seven Epstein-Barr virus-immortalized lymphoblastoid lines generated from FAP patients. The SW480 cell line contains only one allele of the APC gene that has a nonsense mutation at codon 1338, which results in a predicted protein size of 147 kDa (2). All seven FAP cell lines contain a normal allele as well as a known truncating mutation of the APC gene (5). The normal APC gene is predicted to encode a protein of 312 kDa, whereas the mutations result in proteins with predicted relative sizes ranging from 27 to 147 kDa. Analysis of protein lysates by standard Western blot techniques following SDS/PAGE failed to detect full-length APC protein. To facilitate detection of large proteins, protein lysates were subjected to electrophoresis through a denaturing agarose gel system, and protein transfer was accomplished by capillary action. This transfer system coupled with enhanced chemiluminescence allowed detection of full-length APC protein. Polyclonal antibodies anti-NAPC and anti-MAPC and monoclonal antibody FE9 all detected an \approx 300-kDa protein in the FAP cell lines, which was absent in the SW480 cell line (examples in Fig. 1). Furthermore, truncated proteins corresponding to the expected sizes were detected in SW480 and four of the FAP lines (Fig. 1). In one case, freshly drawn blood was obtained from an FAP patient, and peripheral mononuclear cells were isolated. Both mutant (117 kDa) and full-length APC protein were detectable, providing evidence that this analysis could be done directly on blood cells. In three of the FAP lymphoblastoid samples, truncated proteins were not detected, even though full-length APC proteins were easily detectable (data not shown). These included lines with nonsense mutations at codon 232, 301, and 625. Three independent cell lysates from each of these cell lines were analyzed with anti-NAPC or FE9 by using a variety of different gel electrophoresis conditions that should have allowed detection of proteins in the size range predicted by the mutations. These results suggest that certain forms of truncated APC are expressed at very low levels relative to full length, probably due to instability of the mutant transcript or protein.

Similar results were obtained with monoclonal antibodies FE9, CF11, and EF4. A fourth monoclonal antibody, CC1, failed to detect endogenous APC on Western blots even though CC1 was able to immunoprecipitate metabolically labeled endogenous APC (data not shown).

Characterization of APC Protein in Human Tumors. We next determined the status of APC protein in human tumors. To avoid problems with contaminating normal tissue, we examined APC protein in human cell lines derived from

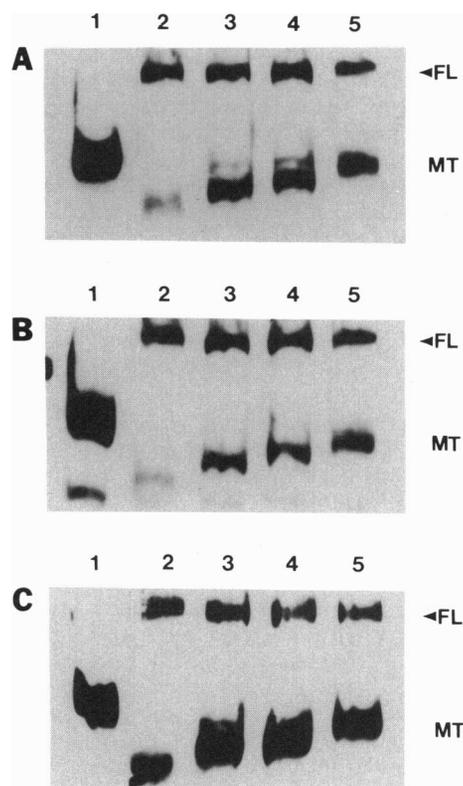


FIG. 1. Detection of APC protein by Western blot analysis. Total protein lysates (100 μ g) were assayed by Western blot with either polyclonal anti-NAPC (A), polyclonal anti-MAPC (B), or monoclonal FE9 (C). Protein lysates were made from the colorectal carcinoma line SW480 (lane 1), which contains a stop codon in APC at 1338, and four different FAP lymphoblastoid cell lines with known truncating APC mutations at codons 1061, 1175, 1211, or 1309, respectively. FL, full-length protein; MT, mutant truncated proteins.

colonic, breast, prostatic, cervical, lung, and pancreatic tumors. A total of 32 colorectal tumor cell lines were studied, including 6 derived from sporadic adenomas, 22 from sporadic carcinomas, 3 from FAP adenomas, and one from an FAP carcinoma. Western blot analysis revealed that 24 (75%) of these lines contained truncated APC protein (examples in Fig. 2). Cell lines were scored positive for truncated APC protein only if the novel band reacted with anti-NAPC, anti-MAPC, and FE9 antibodies. Interestingly, >80% (27 out of 32) of the colon lines were totally devoid of full-length APC protein.

In contrast, seven breast, six prostatic, nine cervical, nine pancreatic, and nine lung carcinoma cell lines each contained only full-length APC protein by Western blot analysis (ex-

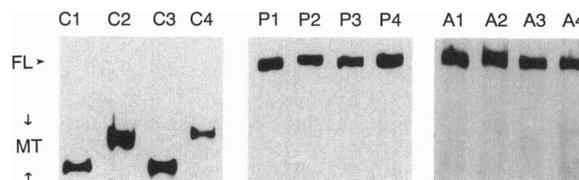


FIG. 2. Characterization of APC protein in tumor cell lines by Western blot analysis. Total protein lysates (100 μ g) were assayed by Western blot with monoclonal antibody FE9. Examples from one colon cancer (C1), three colon adenoma (C2–C4), four prostatic cancer (lanes P1–P4), and four pancreatic cancer (lanes A1–A4) cell lines are shown. FL, full-length protein; MT, mutant truncated protein. The truncated proteins migrate as 100-, 130-, 100-, and 135-kDa polypeptides (left to right) as compared to standard molecular size markers.

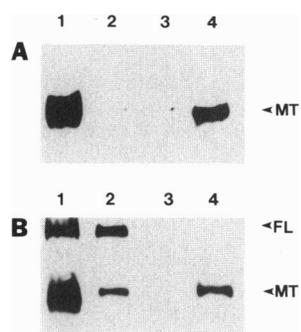


FIG. 3. Subcellular fractionation of normal and mutant APC protein. Cell lines were fractionated as described in *Materials and Methods*, and each fraction was analyzed by Western blot using polyclonal anti-APC antibodies. (A) SW480 cells. (B) Lymphoblastoid cell line derived from an FAP patient with a truncating mutation at codon 1309 of APC. For both A and B, equivalent proportions of the total protein lysate (lane 1), the $100,000 \times g$ insoluble membrane fraction (lane 2), the nuclear fraction (lane 3), and the cytoplasmic fraction (lane 4) were loaded. FL, full-length protein; MT, mutant truncated protein.

amples in Fig. 2). These results suggest that APC mutations might not play a role in the development of these tumors, although it is possible that missense APC mutations and/or very early truncating mutations occur in these tumor types. These results also indicate that the APC protein is expressed at detectable levels in many different tissue types: specifically, lymphoid, lung, breast, prostate, pancreas, and cervix.

Subcellular Localization of APC Protein. Several experiments were undertaken to localize APC at the subcellular level. When cell fractionation using SW480 cells was performed, the truncated protein was found exclusively in the cytosolic fraction (Fig. 3A, lane 4). When an FAP lymphoblastoid line containing full-length and truncated APC protein was fractionated, the results were unexpected. The full-length APC protein was found in the $100,000 \times g$ insoluble membrane fraction while the truncated form was present in the $100,000 \times g$ fraction and the cytosolic fraction (Fig. 3B, lanes 2 and 4). APC fractionation was unaffected by Triton X-100 treatment, but deoxycholate, a weak ionic detergent, completely solubilized full-length and truncated APC (data not shown). This suggested that APC was not a membrane protein, but rather that the full-length APC protein was complexed in an insoluble aggregate. This pattern of fractionation was reproduced with a second FAP lymphoblastoid line containing a different truncated APC protein.

To further define the subcellular localization of APC, immunohistochemical studies were performed on frozen normal colonic mucosa sections by using affinity-purified polyclonal antibodies. Cytoplasmic staining concentrated at the basolateral portion of crypt epithelial cells was seen with anti-NAPC (Fig. 4 A and B) but not with a 3-fold higher concentration of normal rabbit IgG (Fig. 4 D and E). When counterstained with 4',6-diamidino-2-phenylindole, the darkest APC-specific staining was shown to be basolateral to the nuclei. The staining of epithelial cells displays a marked increase from the base of the crypt to the luminal surface. The identical pattern was observed with polyclonal anti-MAPC antibodies. To confirm that this antibody binding was specific for APC, competitions were performed with soluble bacterial GST-NAPC fusion protein or a control unrelated fusion protein (GST-MDM2). The signal was fully blocked by competition with the NAPC fusion protein (Fig. 4F) but was unaffected by a 3-fold higher concentration of the MDM2 fusion protein (Fig. 4C). The signal seen with anti-MAPC antibodies could not be blocked by competition with soluble bacterial NAPC fusion protein, as expected. The same pattern of staining was observed in FAP colonic mucosa from two individuals with either a codon 625 or 1309 truncating mutation (data not shown).

DISCUSSION

Polyclonal and monoclonal antibodies specific to the APC protein were produced and used to characterize mutant and normal forms of the APC protein. Normal APC is an ≈ 300 -kDa protein without any obvious membrane or nuclear localization signals. Consistent with this, immunohistochemical analysis suggests that APC protein is cytoplasmic and concentrated in the basolateral portion of the crypt epithelial cells. APC staining gradually increases as epithelial cells progress from the base of the crypt to the luminal surface. This suggests an association between APC expression and maturation of colonic epithelial cells.

Cell fractionation experiments also suggest that truncated and full-length APC proteins are cytoplasmic but that full-length proteins are present as insoluble aggregates under the conditions used for fractionation. In a cell line devoid of full-length APC (SW480), the mutant APC protein was completely soluble under the same conditions. Yet, in lympho-

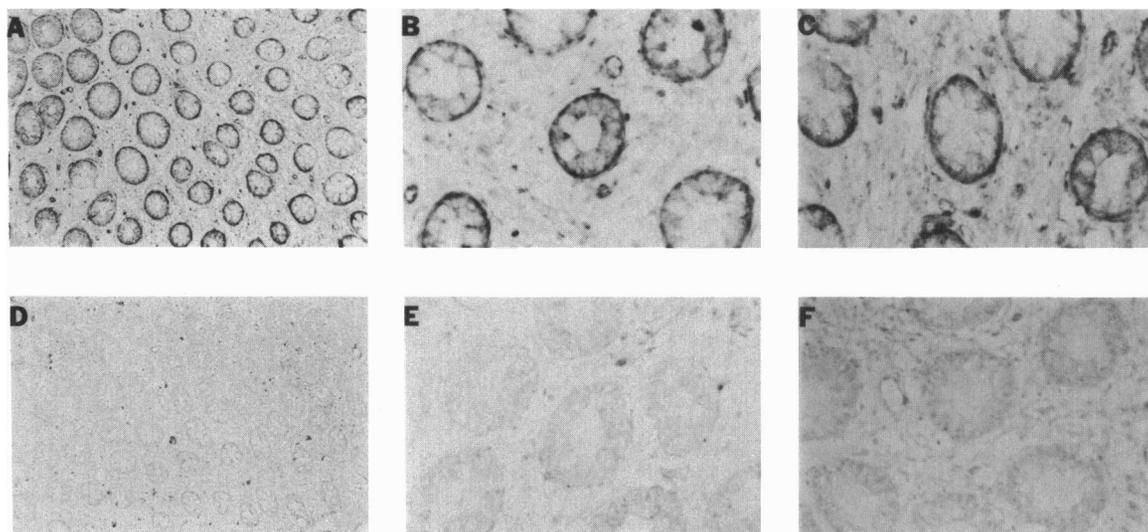


FIG. 4. Immunohistochemistry of normal colonic mucosa using affinity-purified polyclonal anti-NAPC antibodies. The pattern of staining with polyclonal anti-NAPC antibody (A, low power; B, medium power) is not seen with a 3-fold higher concentration of normal rabbit immunoglobulin (D, low power; E, medium power). This signal was effectively blocked by competition with GST-NAPC (F, medium power) but not with a 3-fold higher concentration of GST-MDM2 (C, medium power).

blastoid cells containing full-length APC, the mutant APC proteins were only partially soluble. This suggests that full-length APC could be binding to truncated APC, partially sequestering it to the insoluble fraction. This is consistent with the fact that APC contains heptad repeats, implicated in protein-protein oligomerization and possibly able to mediate the formation of insoluble aggregates.

Using our protein-based assay system, truncated APC protein was detected in several FAP samples and in most colorectal tumor cell lines. In 15 of 16 of the colon lines with detectable truncated protein, the presence of a truncating APC gene mutation was confirmed by using a DNA-based assay (K.J.S. and Steve Powell, unpublished results). These observations are consistent with previous DNA-based analyses of primary colorectal tumors, indicating that at least 60% of colorectal tumors contain truncating APC mutations (7, 8). We also observed that >80% of the colon tumor cell lines were totally devoid of full-length APC protein. This is higher than expected from previous DNA-based analyses, although some tumors have been shown to harbor mutations of both alleles of APC (8). Allelic loss data have implicated a tumor suppressor gene on chromosome 5q in the development of other cancers (22–24). However, APC protein analyses of 40 cell lines representing five noncolonic tumor types failed to identify any truncated APC protein. This suggests that APC mutations do not play a major role in the development of these tumors, although it is possible that these tumor types inactivate APC by other mechanisms (e.g., missense mutation). This contrasts with findings on the tumor suppressor gene, p53, which is inactivated in many different tumor types through the same type of mutation (reviewed in ref. 25). It is also interesting that tumors in FAP patients are mostly confined to colon epithelial cells, even though the present results demonstrate that normal APC protein is expressed in a variety of cell types and the expression of mutant APC is not limited to colonic cells.

Truncated APC protein could not be detected in some FAP samples with known truncating mutations. All of the truncated forms that were not detected were predicted to be smaller than 80 kDa. Other studies have indicated that truncating nonsense mutations can lead to decreased mRNA abundance (26). Also, some abnormal RB tumor suppressor gene transcripts predicted to exist in the lymphoid cells of patients with hereditary retinoblastoma could not be identified (27), raising the possibility that very premature termination of APC mRNA translation could lead to decreased mRNA stability. Alternatively, the short truncated proteins might be unstable. Despite the inability to detect some mutant forms of the APC protein in FAP samples, a significant fraction of FAP mutations can be identified by direct analysis of the APC protein. From the previous mutational analysis in 150 FAP patients, we would predict that at least 35% of FAP patients should have truncated APC protein ranging in size from 90 to 180 kDa and thus detectable by Western blot analysis (5, 6). This should allow simple presymptomatic diagnosis in a significant fraction of FAP kindreds.

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- Kinzler, K. W., Nilbert, M. C., Su, L.-K., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hedge, P., McKechnie, D., Finniear, R., Markham, A., Groffen, J., Boguski, M. S., Altschul, S. F., Horii, A., Ando, H., Miyoshi, Y., Miki, Y., Nishisho, I. & Nakamura, Y. (1991) *Science* **253**, 661–665.
- Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S., Hedge, P., Markham, A., Krush, A. J., Petersen, G., Hamilton, S. R., Nilbert, M. C., Levy, D. B., Bryan, T. M., Preisinger, A. C., Smith, K. J., Su, L.-K., Kinzler, K. W. & Vogelstein, B. (1991) *Science* **253**, 665–669.
- Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., Le Paslier, D., Abderrahim, H., Cohen, D., Leppert, M. & White, R. (1991) *Cell* **66**, 589–600.
- Joslyn, G., Carlson, M., Thliveris, A., Albertsen, H., Gelbert, L., Samowitz, W., Groden, J., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., Le Paslier, D., Abderrahim, H., Cohen, D., Leppert, M. & White, R. (1991) *Cell* **66**, 601–613.
- Miyoshi, Y., Ando, H., Nagase, H., Nishisho, I., Horii, A., Miki, Y., Mori, T., Utsunomiya, J., Baba, S., Petersen, G., Hamilton, S. R., Kinzler, K. W., Vogelstein, B. & Nakamura, Y. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4452–4456.
- Nagase, H., Miyoshi, Y., Horii, A., Aoki, T., Petersen, G., Vogelstein, B., Payne, S., Ogawa, M., Masakazu, M., Nakajima, T., Utsunomiya, J., Baba, S. & Nakamura, Y. (1993) *Hum. Mutat.*, in press.
- Miyoshi, Y., Nagase, H., Ando, H., Horii, A., Ichii, S., Nakatsuru, S., Aoki, T., Miki, Y., Mori, T. & Nakamura, Y. (1992) *Hum. Mol. Genet.* **1**, 229–233.
- Powell, S. M., Nilz, N., Beazer-Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., Vogelstein, B. & Kinzler, K. W. (1992) *Nature (London)* **359**, 235–237.
- Liebovitz, A., Stinson, J. C., McCombs, W. B., McCoy, C. E., Mazur, K. C. & Mabry, N. D. (1976) *Cancer Res.* **36**, 4562–4569.
- McBain, J., Weese, J., Meisner, L., Wolberg, W. & Willson, J. (1984) *Cancer Res.* **44**, 5813–5821.
- Willson, J., Bittner, G., Oberley, T., Meisner, G. & Weese, J. (1987) *Cancer Res.* **47**, 2704–2713.
- Paraskeva, C., Buckle, B. G., Sheer, D. & Wigley, C. B. (1984) *Int. J. Cancer* **34**, 49–56.
- Paraskeva, C., Finerty, S., Mountford, R. A. & Powell, S. C. (1989) *Cancer Res.* **49**, 1282–1286.
- Williams, A. C., Harper, S. J., Marshall, C. J., Gill, R. W., Mountford, R. A. & Paraskeva, C. (1992) *Int. J. Cancer* **52**, 785–790.
- Koerner, T. J., Hill, J. E., Myers, A. M. & Tzagoloff, A. (1991) *Methods Enzymol.* **194**, 477–490.
- Baker, T. A., Grossman, A. D. & Gorss, C. A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6779–6783.
- McKenzie, S. J., Marks, P. J., Lam, T., Morgan, J., Panicali, D. L., Trimpe, K. L. & Carney, W. P. (1989) *Oncogene* **4**, 543–548.
- Smith, D. B. & Johnson, K. S. (1988) *Gene* **67**, 31–40.
- Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L. & Vogelstein, B. V. (1992) *Nature (London)* **358**, 80–83.
- Sheffield, J. N., Graff, D. & Li, H. P. (1987) *Anal. Biochem.* **166**, 49–54.
- Sprott, S. C., Hammond, D. & Savage, N. (1991) *Anal. Biochem.* **194**, 407–412.
- Ashton-Rickardt, P. G., Wyllie, A. H., Bird, C., Dunlop, M. G., Steel, C. M., Morris, R. G., Piris, J., Romanowski, P., Wood, R., White, R. & Nakamura, Y. (1991) *Oncogene* **6**, 1881–1886.
- D'Amico, D., Carbone, D. P., Johnson, B. E., Meltzer, S. J. & Minna, J. D. (1992) *Cancer Res.* **52**, 1996–1999.
- Boynton, R. F., Blount, P. L., Yin, J., Brown, V. L., Huang, Y., Tong, Y., McDaniel, T., Newkirk, C., Resau, J. H., Raskind, W. H., Haggitt, R. C., Reid, B. J. & Meltzer, S. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3385–3388.
- Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. (1991) *Science* **253**, 49–53.
- Lim, S.-K., Sigmund, C. D., Gorss, K. W. & Maquat, L. E. (1992) *Mol. Cell. Biol.* **12**, 1149–1161.
- Dunn, J. M., Phillips, R. A., Zhu, X., Becker, A. & Gallie, B. L. (1989) *Mol. Cell. Biol.* **9**, 4596–4604.