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Apoptosis and *APC* in colorectal tumorigenesis

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ABSTRACT **Tumors result from disruptions in the homeostatic mechanisms that regulate cell birth and cell death. In colon cancer, one of the earliest manifestation of this imbalance is the formation of polyps, caused by somatic and inherited mutations of the adenomatous polyposis coli (*APC*) tumor suppressor gene in both humans and mice. While the importance of APC in tumorigenesis is well documented, how it functions to prevent tumors remains a mystery. Using a novel inducible expression system, we show that expression of *APC* in human colorectal cancer cells containing endogenous inactive *APC* alleles results in a substantial diminution of cell growth. Further evaluation demonstrated that this was due to the induction of cell death through apoptosis. These results suggest that apoptosis plays a role not only in advanced tumors but also at the very earliest stages of neoplasia.**

Tumors result from an imbalance between cell birth and cell death. However, few genes affecting cell death have been identified in common human epithelial cancers, and the best studied of these, p53, seems to generally function only in the late stages of tumorigenesis (1–5). Mutations of the adenomatous polyposis coli (*APC*) gene are associated with the earliest stages of colorectal tumorigenesis. Humans who inherit a germline *APC* mutation develop hundreds of benign colorectal tumors, some of which progress to cancer (6–9). Likewise, mice who inherit mutant *APC* develop multiple intestinal tumors (10, 11). Somatic mutations of *APC* also appear to be responsible for the initiation of colorectal cancers in the general population as they are found in small adenomas and dysplastic microscopic lesions (12–15). At the biochemical level, several properties of *APC* have been described, including homooligomerization (16, 17) and binding to β-catenin (18, 19), EB1 (20), and the microtubule cytoskeleton (21, 22). At the functional level, however, little is known about how *APC* operates to prevent colorectal tumorigenesis. In this report, we evaluate the effects of restoring *APC* in human colorectal cancer cells.

MATERIALS AND METHODS

Cell Culture. The colon carcinoma cell line HT-29 was obtained from the American Type Culture Collection and cultivated in McCoy's 5A media (GIBCO/BRL) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin.

Vector Construction and Transfection. The pSAR-MT inducible expression plasmid was constructed as follows. Two oligonucleotides (5′-GATCTCGAGCTCCCTGCA-3′ and 5′-GGGAGCTCGA-3′) were annealed, and the resulting fragment was cloned into the *Bam*HI and *Pst*I sites of the scaffold attachment region (SAR)-containing plasmid p8 (23). The resulting plasmid, pJM7, consisted of two SAR sequences flanking a short polylinker containing restriction sites for *Xho*I and *Pst*I. The mutant metallothionein (MT) promoter (24) was amplified by PCR and cloned into pCEP4 (Invitrogen) linearized with *Bgl*II and *Not*I, yielding pCEP-MT. A *Sal*I–*Bam*HI fragment containing an intron from the globin gene was then inserted into the *Xho*I and *Bam*HI sites of pCEP-MT, yielding pCEP-MTi. Finally, the MT promoter/intron/poly(A) region was removed from pCEP-MTi by digestion with *Sal*I and cloned into the *Xho*I site of pJM7, yielding pSAR-MT, as shown in Fig. 1*A*. The entire coding regions of *APC* [from pCMV-APC (22)] and β-galactosidase (from pCMVβ, CLONTECH) were inserted into the *Bam*H1 site of pSAR-MT to make pSAR-MT-APC and pSAR-MT-β-gal, respectively. Lipofectin (GIBCO/BRL) was used to transfect HT-29 cells with the linearized pSAR-MT expression vectors (5.0 μg) and the hygromycin resistance vector pCEP4 (0.5 μg, Invitrogen). After selection with 0.6 mg/ml hygromycin, clones were isolated and expanded. HT29-APC1 and HT29-APC2 were identified after screening 250 clones for inducible full-length APC expression by Western blotting. Unless otherwise indicated, cells were induced with 100 μM $ZnCl_2$ for the times indicated.

Immunoprecipitations and Western Blotting. For immunoprecipitation, cells were lysed in MEBC lysis buffer with protease and phosphatase inhibitors as described (19) and equalized for the amount of protein. The immunoprecipitation and Western blots were performed essentially as described (19, 20, 25). The following monoclonal antibodies were used: FE-9 and IE1 (Oncogene Science) for the amino and carboxyl termini of APC, respectively; EA3 (Oncogene Science) for EB1; and β-cat (Transduction Laboratories, Lexington, KY) for β-catenin. For Western analysis of APC expression, cells were lysed by adding Laemmli sample buffer directly to the plate and analyzed as described (25).

Flow Cytometry and Hoechst Staining. Attached and floating cells were harvested and resuspended in 50 μl of PBS and 350 μl of staining solution containing 0.6% Nonidet P-40 and 3.7% formaldehyde, and 0.01 mg/ml Hoechst 33258 was added. Cells were then analyzed by flow cytometry as described (26). For Hoechst staining, cells were prepared as for flow cytometry analysis and photographed under fluorescence microscopy.

Terminal Deoxytransferase-Mediated Deoxyuridine Nick End-Labeling (TUNEL) Assay. Attached cells were fixed for 30 min with 1% paraformaldehyde in PBS and permeabilized with 1% Nonidet P-40. Fixed cells were then incubated for 1 hr in terminal deoxynucleotidyl transferase buffer (United States Biochemical) supplemented with 150 mM NaCl, 5 μM biotin-16-dUTP (Boehringer Mannheim), and 50 units/ml terminal deoxynucleotidyl transferase buffer. Cells were washed with PBS and positive cells visualized using the Vectastain kit (Vector Laboratories) with diaminobenzidine tetrahydrochloride staining.

RESULTS

HT-29 is a colorectal cancer cell line widely used for experimental studies because it retains many biochemical and phys-

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Abbreviations: APC, adenomatous polyposis coli; NSAID, nonsteroidal antiinflammatory drug; SAR, scaffold attachment region; TUNEL, terminal deoxytransferase-mediated deoxyuridine nick end-labeling; MT, metallothionein.

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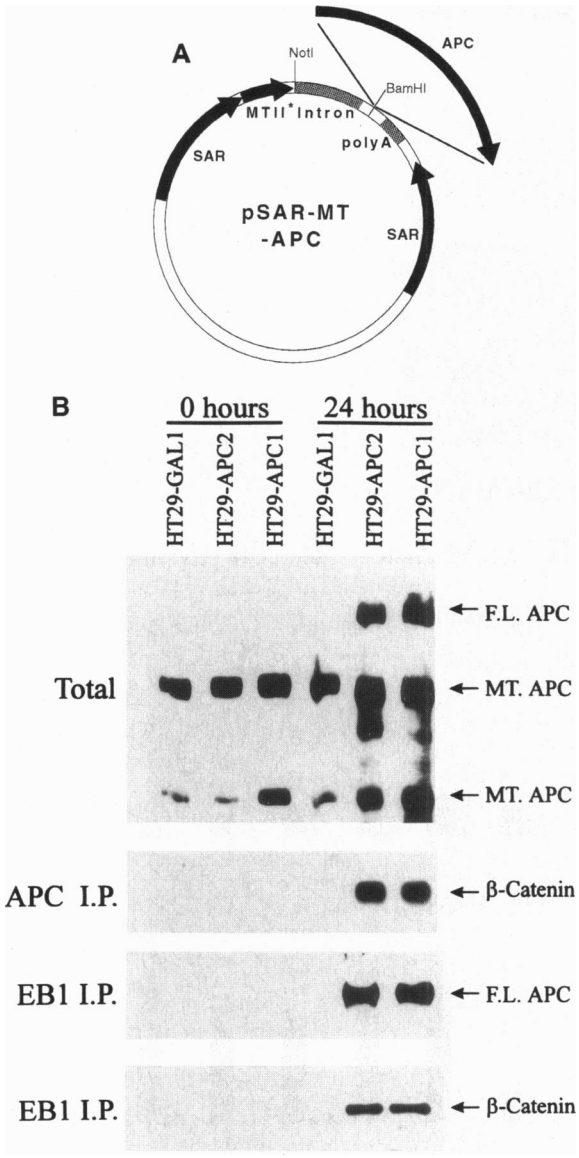

FIG. 1. Expression of APC in HT-29 cells. (*A*) The pSAR-MT vector was constructed to allow stable inducible expression of APC in mammalian cells. Features of the pSAR vector include SARs (23), a modified MT promoter (MTII*) (24), splice donor and acceptor sites (intron), and a polyadenylation site [poly(A)]. HT-29 cells were transfected with pSAR-MT-APC, and the status of APC in selected clones was determined. (*B*) Two cell lines, HT29-APC1 and HT29-APC2, demonstrated inducible expression of APC when treated with zinc. Western blot analysis with an antibody specific to the amino terminus of APC (total) detected full-length APC protein (F.L. APC) in both HT29-APC1 and HT29-APC2 after induction (24 hr). In contrast, only the endogenous mutant APC (MT. APC) could be detected before induction (0 hr) and in the control line HT29-GAL1. Immunoprecipitations with antibodies specific for the carboxyl terminus of APC (APC I.P.) or the APC-associated protein EB1 (EB1 I.P.) were performed on HT29-GAL1, HT29-APC1, and HT29-APC2 before (0 hr) and after (24 hr) induction. The resulting precipitates were analyzed by Western blot analysis with antibodies specific to β-catenin (β-catenin) or APC (F.L. APC). The immunoprecipitations demonstrate that the induced full-length APC protein directly interacts with β-catenin and EB1 and that both EB1 and β-catenin can simultaneously bind the same full-length APC protein.

iologic features of normal colorectal epithelial cells (27). As do the great majority of colorectal cancer cell lines (25), HT-29 contains no intact APC protein; instead, two carboxyl-terminal-truncated APC proteins of approximately 100 kDa and 200 kDa are present. Efforts to achieve stable expression of full-length APC in this and other colorectal cancer lines containing mutant APC were uniformly unsuccessful (ref. 28; unpublished data). We assumed that continuous *APC* expression was inhibitory to cell growth and therefore attempted to use inducible expression vectors to examine the effect of APC. Standard inducible expression vectors did not yield the expected expression patterns, and we therefore engineered a new vector incorporating the following two features: (*i*) an MT-derived promoter with very low basal activity in the absence of zinc (24) and (*ii*) SARs (23) flanking the *APC* gene regulatory elements. SARs increase the level of expression of stably transfected genes (23). The vector (pSAR-MT) incorporating these and other features is illustrated in Fig. 1*A*. *APC* cDNA was inserted into pSAR-MT to derive pSAR-MT-APC, and clones of HT-29 cells transfected with this construct were isolated and evaluated for APC expression. Two of 250 clones were found to express APC after 12 hr of zinc induction and maintain such expression for at least 4 days (clones HT29-APC1 and HT29-APC2). Following 24 hr of induction, the concentration of exogenous, full-length APC protein was comparable to the level of endogenous, mutant APC protein (Fig. 1*B*, total). Clones stably transfected with pSAR-MT incorporating a β-galactosidase cDNA insert were used as controls.

Little is known about the biochemical properties of APC, though the protein has been shown to bind to two intracellular proteins, β-catenin (18, 19) and EB1 (20). β-catenin transmits signals from the E-cadherin adhesion proteins to the cell's interior (29–31), while the function of EB1 is not known. To show that the induced APC protein functioned as expected in clones HT29-APC1 and HT29-APC2, immunoprecipitation experiments were performed. First, cell lysates were immunoprecipitated with monoclonal antibodies specific for the carboxyl terminus of APC and the immunoprecipitates examined for the presence of β-catenin via Western blotting. The induced full-length APC protein was associated with β-catenin in clones HT29-APC1 and HT29-APC2 (Fig. 1*B*, APC I.P.). The cell lysates were also immunoprecipitated with monoclonal EB1 antibodies and examined for the presence of full-length APC and β-catenin. Both full-length APC and β-catenin were found associated with EB1 in the *APC*-expressing clones HT29-APC1 and HT29-APC2 (Fig. 1*B*, EB1 I.P.). This experiment demonstrates for the first time that EB1 and β-catenin can be simultaneously bound to full-length APC *in vivo*, suggesting that they may function in the same pathway. We found no evidence that APC sequestered or down-regulated either β-catenin or EB1, as the β-catenin-E-cadherin complexes and the steady-state levels of β-catenin, E-cadherin, and EB1 were not significantly altered after full-length APC induction (data not shown).

We next evaluated the growth properties of the various clones. The first observation was that clone HT29-APC1 did not reach confluence in the presence of induction, even after long periods of culture, while zinc had no apparent effect on cell density of the β-galactosidase control clone (Fig. 2*A*). As expected, both clones reached confluency in the absence of induction. To ensure that this effect was specific for APC induction, 40 additional clones obtained through transfection with the pSAR-MT-GAL construct were examined; none showed any change in growth in the presence of zinc. Moreover, clones obtained through *APC* transfection but expressing only truncated APC proteins also grew as well as the control clones. These observations were confirmed and extended by examining the growth kinetics of HT29-GAL1, HT29-APC1, and HT29-APC2 in the presence and absence of induction (Fig. 2*B*). In contrast to what was observed following transient high level expression of APC in a murine fibroblast cell line containing endogenous wild-type APC (32), flow cytometric analysis of the induced HT-29 cells revealed that the APC-induced clones were not blocked in any specific phase of the

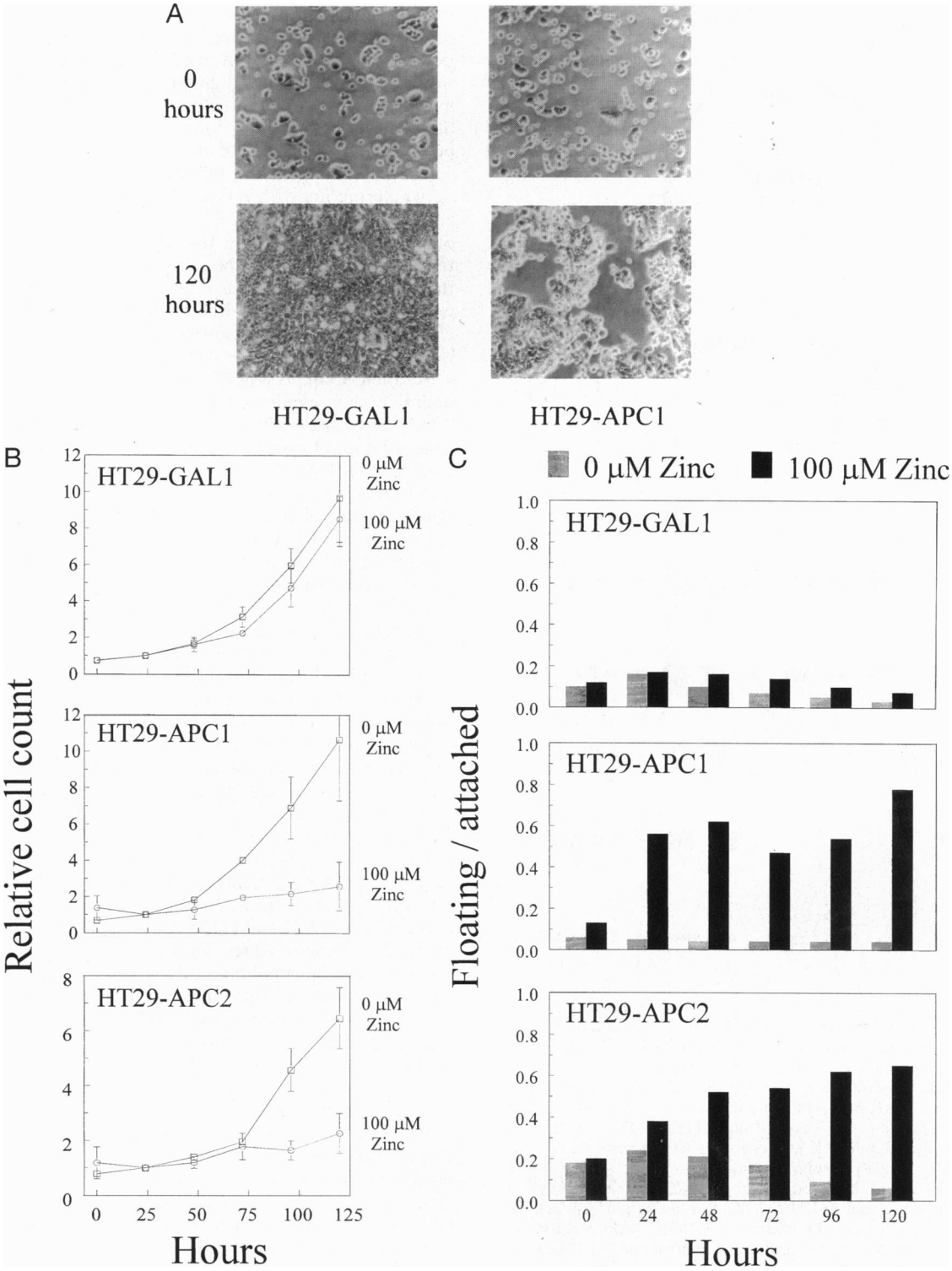

FIG. 2. Expression of full-length APC inhibits growth. (*A*) cell lines HT29-APC1 and HT29-GAL1 were photographed after 0 hr or 120 hr of induction with 100 μM zinc. HT29-APC1 cells failed to become confluent even after 120 hr. (*B*) The growth rates of HT29-APC1, HT29-APC2, and HT29-GAL1 cells were determined by counting attached cells at various time points (0–120 hr) in the presence (100 μM zinc) or absence (0 μM zinc) of induction. The results reported are the average of five experiments for HT29-GAL1 and HT29-APC1 and three experiments for HT29-APC2. The number of cells was normalized to the 24-hr time point to allow consolidation of the different experiments. (*C*) The ratio of floating to attached cells was determined for each time point in the presence (100 μM zinc) or absence (0 μM zinc) of induction for a representative experiment. APC expression greatly increased this ratio in both clones tested, suggesting increased cell death.

cell cycle, as might have been expected had their growth been inhibited by cyclin-dependent kinase inhibitors or other proteins that primarily disrupt cell cycle control mechanisms (Fig. 3). Instead, the only abnormality observed was an increase in cells containing a sub-G1 DNA content. This profile often indicates that apoptosis has taken place (33). This was consistent with the morphological changes observed in the cells by phase contrast microscopy (Fig. 2*A*). Instead of dividing and forming confluent cellular lawns, as did the control cells, APC-expressing cells gradually rounded up and detached (Fig.

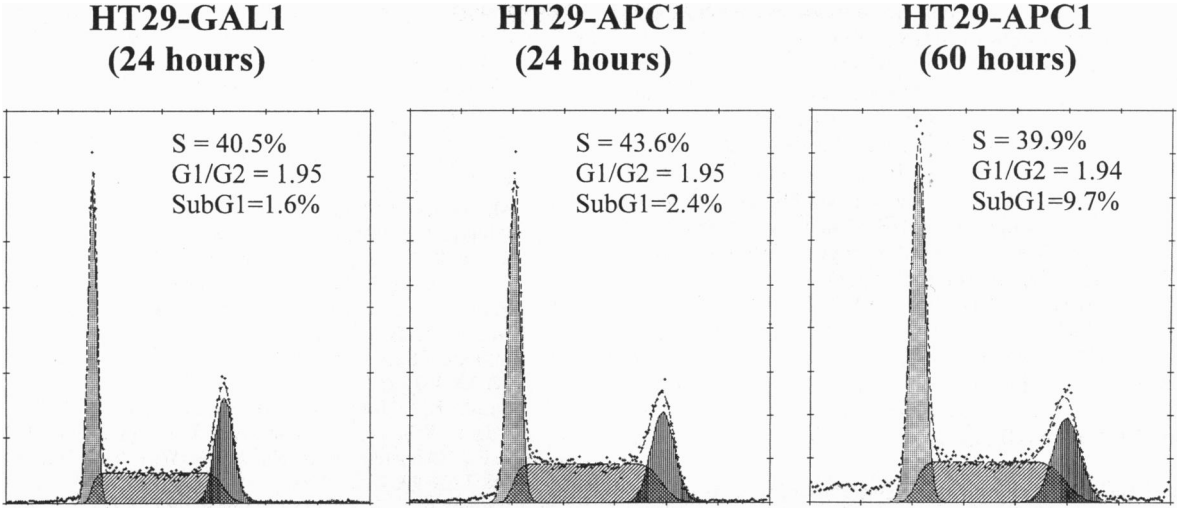

FIG. 3. Flow cytometric analysis of cells expressing full-length APC. Flow cytometric analysis was performed on HT29-GAL1 cells 24 hr after induction and on HT29-APC1 cells 24 hr and 60 hr after induction. Cell count and DNA content are represented by the ordinate and abcissa, respectively. The raw data are indicated by the dotted line and the G1, S, and G2 fractions were shaded and quantitated using the MULTICYCLE software package. Data to the left of the the G1 (leftmost) shaded area represents the cells with lower (sub-G1) DNA content. While the fraction of cells in S phase and the G1/G2 ratio seemed undisturbed after induction of full-length APC expression, there was a marked increase in sub-G1 cells after induction.

2*C*). This process began at 24 hr following induction, and by 48 hr, substantial numbers of cells were found floating in the culture medium (Fig. 2*C*). These morphological changes were correlated with the levels of full-length APC protein. When APC induction was incomplete due to the deliberate addition of a suboptimal concentration of zinc (50 μM instead of the usual 100 μM), growth inhibition was not observed (data not shown).

Two DNA-based assays were then used to confirm that the APC-expressing cells were indeed undergoing apoptosis. Staining of the floating cells from APC-expressing clones with the DNA-binding dye Hoechst 33258 demonstrated brightly fluorescent condensed chromatin or micronuclei, indicating that the excess floating cells in the induced clones had undergone apoptosis (Fig. 4*A*) (33). These hallmarks of apoptosis were present in virtually every floating cell from APC-induced samples. Apoptosis was further assessed on attached cells using the TUNEL assay. The proportion of TUNEL positive cells ranged from 0.1% to 0.4% in uninduced HT29-GAL1 and HT29-APC1. After induction, HT29-APC1 showed a 10-fold increase to 3% positive cells, whereas only 0.4% of HT29-GAL1 cells were positive (Fig. 4*B*; data not shown).

DISCUSSION

Taken together, these results demonstrate that the expression of APC in HT29 cells inhibits cell growth through increased apoptosis. The kinetics of this process explain why induction of APC is incompatible with net cell growth in the induced cells (Fig. 2) and explain the failure to obtain clonal growth of cells stably expressing APC following transfection with appropriate vectors. Immunohistochemical experiments have shown that APC is expressed in normal colorectal epithelial cells as they migrate toward the top of the crypt (25, 35). Our data suggest that this expression is likely to result in apoptosis of the migrating cells, a process critical for achieving cellular equilibrium in actively regenerating tissues such as colonic mucosa (36). Disruption of APC would presumably disturb the equilibrium between new cell formation at the base of the crypts and cell death at the top of the crypts, leading to a relative expansion (tumor) of the progeny of *APC*-mutant cells.

The apoptosis we observed is independent of p53, as HT-29 cells, like most colorectal cancer cells, do not contain functional p53 protein (1, 37). However, p53 mutations generally occur late in colorectal tumorigenesis, near the transition to malignancy (1, 2). This suggests that multiple apoptotic controls exist to control neoplasia in long-lived organisms such as the human. In colorectal mucosa, the first apoptotic control point appears to be mediated by APC, while a reserve system,

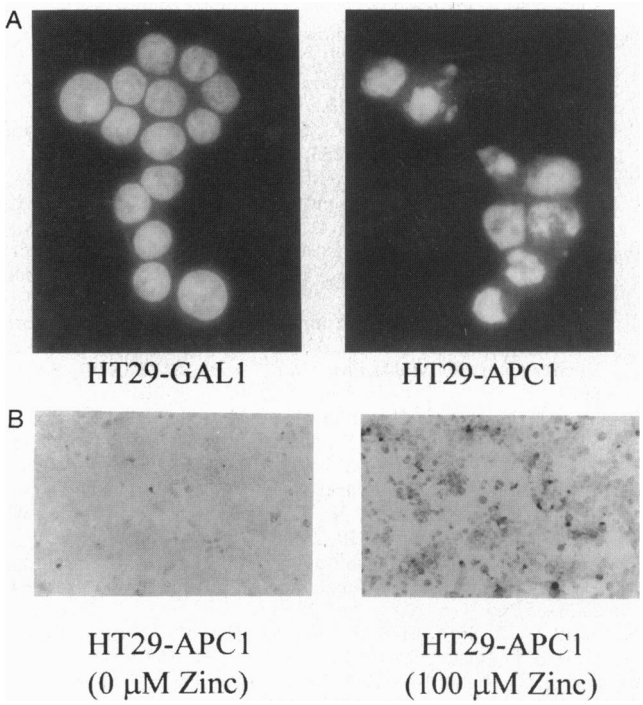

FIG. 4. Cells expressing full-length APC show increased apoptosis. (*A*) After 60 hr of induction, floating HT29-APC1 cells were harvested, stained with Hoechst 33258 dye, and photographed under fluorescence microscopy. Nuclei of HT29-APC1 clearly show the fragmentation patterns indicative of apoptosis. For comparison, healthy attached HT29-GAL1 cells are shown following zinc induction. (*B*) Apoptosis in attached HT29-APC1 cells was examined using the TUNEL assay (34). Only 0.3% of uninduced cells (0 μM zinc) stained positive by TUNEL, while 3% were positive after 88 hr after zinc induction (100 μM zinc).

required only under exceptional circumstances, such as the peculiar microenvironment of tumors, is controlled by p53 (4).

Finally, our results are relevant to previous observations about cancer chemotherapeutic agents. It has been shown that certain nonsteroidal antiinflammatory drugs (NSAIDs) can reduce the size and number of colorectal tumors in genetically predisposed individuals (38–42). Because colorectal tumor cells lack intact APC, it was suggested that these NSAIDs replace a physiologic function of *APC* that was abrogated by mutation. Interestingly, it was recently suggested that Sulindac, the prototype NSAID, exerts its antineoplastic activity by promoting apoptosis (43–46). Our finding that APC expression specifically induces apoptosis suggests that the cellular function replaced by NSAIDs is the induction of apoptosis. These data have obvious implications for the identification of other chemopreventative agents that might be useful for patients at risk for this disease.

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1. Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K., Hamilton, S. & Vogelstein, B. (1990) *Cancer Res.* **50,** 7717–7722.
2. Purdie, C. A., O'Grady, J., Piris, J., Wyllie, A. H. & Bird, C. C. (1991) *Am. J. Pathol.* **138,** 807–813.
3. Sidransky, D., Frost, P., Von Eschenbach, A., Oyasu, R., Preisinger, A. C. & Vogelstein, B. (1992) *N. Engl. J. Med.* **326,** 737–740.
4. Graeber, T. G., Osmanian, C., Jacks, T., Housman, D. E., Koch, C. J., Lowe, S. W. & Giaccia, A. J. (1996) *Nature (London)* **379,** 88–91.
5. Kovach, J. S., Hartman, A., Blaszyk, H., Cunningham, J., Schaid, D. & Sommer, S. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1093–1096.
6. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., *et al.* (1991) *Cell* **66,** 589–600.
7. Joslyn, G., Carlson, M., Thliveris, A., Albertsen, H., Gelbert, L., *et al.* (1991) *Cell* **66,** 601–613.
8. Kinzler, K. W., Nilbert, M. C., Su, L. K., Vogelstein, B., Bryan, T. M., *et al.* (1991) *Science* **253,** 661–665.
9. Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S. & Hedge, P. (1991) *Science* **253,** 665–669.
10. Su, L. K., Kinzler, K. W., Vogelstein, B., Preisinger, A. C., Moser, A. R., Luongo, C., Gould, K. A. & Dove, W. F. (1992) *Science* **256,** 668–670.
11. Fodde, R., Edelmann, W., Yang, K., van Leeuwen, C., Carlson, C., Renault, B., Breukel, C., Alt, E., Lipkin, M., Khan, P. M. & Kucherlapati, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8969–8973.
12. 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.
13. Powell, S. M., Zilz, N., Beazer-Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., Vogelstein, B. & Kinzler, K. W. (1992) *Nature (London)* **359,** 235–237.
14. Jen, J., Powell, S. M., Papadopoulos, N., Smith, K. J., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1994) *Cancer Res.* **54,** 5523–5526.
15. Smith, A. J., Stern, H. S., Penner, M., Hay, K., Mitri, A., Bapat, B. V. & Gallinger, S. (1994) *Cancer Res.* **54,** 5527–5530.
16. Joslyn, G., Richardson, D. S., White, R. & Alber, T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11109–11113.
17. Su, L. K., Johnson, K. A., Smith, K. J., Hill, D. E., Vogelstein, B. & Kinzler, K. W. (1993) *Cancer Res.* **53,** 2728–2731.
18. Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S. & Polakis, P. (1993) *Science* **262,** 1731–1734.
19. Su, L. K., Vogelstein, B. & Kinzler, K. W. (1993) *Science* **262,** 1734–1737.
20. Su, L. K., Burrell, M., Hill, D. E., Gyuris, J., Brent, R., Wiltshire, R., Trent, J., Vogelstein, B. & Kinzler, K. W. (1995) *Cancer Res.* **55,** 2972–2977.
21. Munemitsu, S., Souza, B., Muller, O., Albert, I., Rubinfeld, B. & Polakis, P. (1994) *Cancer Res.* **54,** 3676–3681.
22. Smith, K. J., Levy, D. B., Maupin, P., Pollard, T. D., Vogelstein, B. & Kinzler, K. W. (1994) *Cancer Res.* **54,** 3672–3675.
23. Poljak, L., Seum, C., Mattioni, T. & Laemmli, U. K. (1994) *Nucleic Acids Res.* **22,** 4386–4394.
24. Makarov, S. S., Jonat, C. & Haskill, S. (1994) *Nucleic Acids Res.* **22,** 1504–1505.
25. Smith, K. J., Johnson, K. A., Bryan, T. M., Hill, D. E., Markowitz, S., Willson, J. K., Paraskeva, C., Petersen, G. M., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2846–2850.
26. Waldman, T., Kinzler, K. & Vogelstein, B. (1995) *Cancer Res.* **55,** 5187–5190.
27. von Kleist, S., Chany, E., Burtin, P., King, M. & Fogh, J. (1975) *J. Natl. Cancer Inst.* **55,** 555–560.
28. Groden, J., Joslyn, G., Samowitz, W., Jones, D., Bhattacharyya, N., Spirio, L., Thliveris, A., Robertson, M., Egan, S., Meuth, M. & White, R. (1995) *Cancer Res.* **55,** 1531–1539.
29. Peyrieras, N., Louvard, D. & Jacob, F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8067–8071.
30. Ozawa, M., Baribault, H. & Kemler, R. (1989) *EMBO J.* **8,** 1711–1717.
31. Kemler, R. (1993) *Trends Genet.* **9,** 317–321.
32. Baeg, G.-H., Matsumine, A., Kuroda, T., Bhattacharjee, R. N., Miyashiro, I., Toyoshima, K. & Akiyama, T. (1995) *EMBO J.* **14,** 5618–5625.
33. Wyllie, A. H. (1995) *Curr. Opin. Genet. Dev.* **5,** 97–104.
34. Gorczyca, W., Gong, J. & Darzynkiewicz, Z. (1993) *Cancer Res.* **53,** 1945–1951.
35. Miyashiro, I., Senda, T., Matsumine, A., Baeg, G. H., Kuroda, T., Shimano, T., Miura, S., Noda, T., Kobayashi, S., Monden, M., Toyoshima, K. & Akiyama, T. (1995) *Oncogene* **11,** 89–96.
36. Hall, P. A., Coates, P. J., Ansari, B. & Hopwood, D. (1994) *J. Cell Sci.* **107,** 3569–3577.
37. Rodrigues, N. R., Rowan, A., Smith, M. E. F., Kerr, U. B., Bodmer, W. F., Gannon, J. V. & Lane, D. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7555–7559.
38. Waddell, W. R., Ganser, G. F., Cerise, E. J. & Loughry, R. W. (1989) *Am. J. Surg.* **157,** 175–179.
39. Labayle, D., Fischer, D., Vielh, P., Drouhin, F., Pariente, A., Bories, C., Duhamel, O., Trousset, M. & Attali, P. (1991) *Gastroenterology* **101,** 635–639.
40. Rigau, J., Pique, J. M., Rubio, E., Planas, R., Tarrech, J. M. & Bordas, J. M. (1991) *Ann. Intern. Med.* **115,** 952–954.
41. Giardiello, F. M., Hamilton, S. R., Krush, A. J., Piantadosi, S., Hylind, L. M., Celano, P., Booker, S. V., Robinson, C. R. & Offerhaus, G. J. (1993) *N. Engl. J. Med.* **328,** 1313–1316.
42. Winde, G., Gumbinger, H. G., Osswald, H., Kemper, F. & Bunte, H. (1993) *Int. J. Colorectal Dis.* **8,** 13–17.
43. Piazza, G. A., Rahm, A. L. K., Krutzsch, M., Sperl, G., Paranka, N. S., Gross, P. H., Brendel, K., Burt, R. W., Alberts, D. S., Pamukcu, R. & Ahnen, D. J. (1995) *Cancer Res.* **55,** 3110–3116.
44. Tsujii, M. & Dubois, R. N. (1995) *Cell* **83,** 493–501.
45. Shiff, S. J., Qiao, L., Tsai, L. L. & Rigas, B. (1995) *J. Clin. Invest.* **96,** 491–503.
46. Pasricha, P. J., Bedi, A., O'Connor, K., Rashid, A., Akhtar, A. J., Zahurak, M. L., Piantadosi, S., Hamilton, S. R. & Giardiello, F. M. (1995) *Gastroenterology* **109,** 994–998.