Growth Phase-dependent Expression of ICAD-L/DD45 Modulates the Pattern of Apoptosis in Human Colonic Cancer Cells

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ABSTRACT

The inhibitor of caspase-3-activated DNase (ICAD) is a caspase-3 substrate that controls nuclear apoptosis. ICAD has two isoforms: a functional isoform of M, 45,000, ICAD-L/DNA fragmentation factor (DFF) 45; and a M, 35,000 isoform, ICAD-S/DF45. ICAD-deficient murine cells display resistance to apoptotic stimuli and absence of typical nuclear changes of apoptosis. Our aim was to: (a) characterize the ICAD expression in several human colonic cancer cell lines compared with human normal colonocytes; and (b) correlate the phenotypic features of apoptosis to the level of ICAD expression. ICAD expression was assessed by immunoblot analysis. Early markers of apoptosis of cultured cells included lactate dehydrogenase retention in dying cells, cytokeratin 18 cleavage, and caspase-3 activation. Nuclear markers of apoptosis were assessed by Hoechst staining of nuclei, electron microscopy, and DNA electrophoresis. Inhibition of caspases was performed using a broad-spectrum caspase inhibitor, z-Val-Ala-Asp-fluoromethyl ketone. ICAD expression was restricted to the functional ICAD-L/DD45 isoform in colon cancer cells as well as in human normal colonocytes. In a clonal derivative of HT29 cells (HT29-Cl.16E cells), ICAD expression was found to be down-regulated during the exponential phase of growth, and the cell death triggered by IFN-γ, anti-Fas antibody plus Adriamycin was characterized by the expression of early markers of apoptosis, whereas the key nuclear features of apoptosis were absent. In contrast, exposure of confluent cells to this treatment led to a typical apoptotic nuclear fragmentation. Both forms of apoptosis, in exponentially growing and confluent cells, were sensitive to the broad spectrum inhibitor of caspases, z-Val-Ala-Asp-fluoromethyl ketone. Our findings support the concept that the expression of ICAD is essential to the execution of full-blown apoptosis in colon cancer cells. Altogether, our results point to ICAD as a potential target for restoring a normal apoptotic signal transduction pathway in colon cancer cells.

INTRODUCTION

Programmed cell death or apoptosis is a form of cell death characterized by distinct morphological and biochemical alterations. Apoptotic cell death occurs in two phases: (a) a commitment to cell death; and (b) an execution phase characterized by condensation and fragmentation of nuclear chromatin associated with internucleosomal cleavage of DNA, recognized as “DNA ladder” on conventional agarose gel electrophoresis. Apoptosis is a physiological process, critical in development and tissue homeostasis including normal cell turnover, regulation of the immune system, or hormone-dependent atrophy (reviewed in Ref. 1). Recent studies indicate that widely used chemotherapeutic agents induce apoptosis in susceptible cells (reviewed in Refs. 2 and 3). Dysregulation of apoptosis provides a growth advantage to cancer cells and might underly the resistance of a variety of tumor cells to external signals, i.e., ligands acting on specific receptors or chemotherapeutic drugs. In this context, it is of utmost importance to decipher the molecular pathways that: (a) integrate the apoptotic stimuli (receptor activation, receptor-proximal molecules); and (b) form the apoptosis execution machinery. Recently, several lines of investigation have highlighted the role of cysteine proteases of the caspase family in the signaling and execution of apoptosis by cleaving critical cellular proteins solely after aspartate residues. Once activated, initiator caspases, in turn, activate the executioner caspases including caspase-3 that promote apoptosis by cleaving cellular substrates that induce morphological and biochemical features of apoptosis (reviewed in Ref. 1). Recent studies have indicated that apoptotic DNA fragmentation and associated nuclear changes are largely attributable to a M, 40,000 nuclease termed CAD3 (4, 5), also known as CPAN (6) or DFF40 (7–9). In nonapoptotic cells, CAD/CPAN/DD40 remains inactive because it is bound to its natural inhibitor ICAD (4–6). ICAD can be expressed as two isoforms, a M, 45,000 isoform (ICAD-L/DD45) and a M, 35,000 isoform (ICAD-S/DF45), caused by alternative splicing (4, 10). Although both ICAD-L/DD45 and ICAD-S/DF45 can bind and inhibit CAD/CPAN/DD40, only ICAD-L/DD45 was reported to be functional because it is able to act as a molecular chaperone for the nuclease to ensure its correct folding (4–6). ICAD is a caspase-3 substrate that must be cleaved to be inactivated and allow DFF40 to execute nuclear internucleosomal DNA fragmentation (4–7). Interestingly, Zhang et al. (11) have shown recently that thymocytes from ICAD-deficient mice (ICAD−/−) are more resistant to several apoptotic stimuli than wild-type control cells. In addition, dying ICAD-deficient cells exhibit different morphologies compared with wild-type control cells, because they show a reduced degree of chromatin condensation and absent nuclear fragmentation (11). Because several cancer cell types including colon cancer cells are characterized by impaired apoptosis, an attractive hypothesis is that ICAD could be down-regulated and that ICAD down-regulation could be correlated with an impaired apoptosis. Here, we report that: (a) ICAD is expressed only as the functional isoform of M, 45,000 in human normal colonocytes and in human colon cancer cell lines; (b) in a clonal derivative of a colon cancer cell line (HT29-Cl.16E cells), ICAD expression is dependent on the phase of growth, i.e., cells in the exponential phase of growth down-regulate ICAD expression; (c) when exposed to apoptotic stimuli, growing cancer cells display early markers of apoptosis, i.e., intracytoplasmic LDH retention, cytokeratin 18 cleavage, and caspase-3 activation, whereas they do not exhibit the nuclear morphological characteristics of late apoptosis; and (d) exposure of confluent cancer cells expressing ICAD to the apoptotic stimuli induce full-blown apoptotic nuclear fragmentation.

MATERIALS AND METHODS

Cell Lines. The colon cancer cell lines SW480, SW1116, SW620, and Caco-2 were purchased from American Type Culture Collection (Rockville, MD). Caco-2 were purchased from American Type Culture Collection (Rockville, MD).
MD), and the HT29 colonic cancer cell line (12) was generously provided by Dr. J. Fogh. The clonal derivatives of the HT29 cell line, HT29-C1.16E and HT29-C1.19A cells (13), differentiate upon reaching confluency into mucous-secreting and ion-transporting colonic epithelial cells, respectively (13–17). The HGT-1 cell line is a human gastric epithelial cell line (18). Jurkat E6.1, obtained from the European Collection of Cell Culture (Salisbury, United Kingdom), was cultured routinely in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% FCS.

Cell Culture for Immunoblot Analysis. For immunoblot analysis, confluent cells were harvested at least 3 days after confluency. For cells in the exponential phase of growth, Caco-2 (20 × 10^5 cells/ml), HTG-1 (20 × 10^5 cells/ml), HTG-2 (40 × 10^5 cells/ml), HTG-2-C1.19A (60 × 10^5 cells/ml), and HTG-2-C1.16E (60 × 10^5 cells/ml) were seeded in 30 ml of DMEM/FCS in 10-cm diameter Petri dishes (Costar, Brumath, France). After 3 days of culture, the medium was discarded and replaced by 6 ml of DMEM/FCS, and cells were submitted or not to a cytotoxic treatment as described below.

Treatment for Cell Death Induction. HT29-C1.16E cells were seeded at 60 × 10^5 cells/ml in 5 ml of DMEM/10% FCS in six-well culture plates (Costar). Three days later, the medium was discarded and replaced by 1 ml of DMEM/10% FCS. Cells were treated with a human activating anti-Fas antibody (10 ng/ml, clone CH-11; Upstate Biotechnology, Euromedex, Marcy-l'Etoile, France) was added to the culture medium for the last 12 h of treatment at the concentrations indicated. This treatment with the three agents is referred to as IFA treatment throughout the text. After treatment, both viable adherent cells and floating dead cells were collected.

Jurkat cells seeded were cultivated at 1 × 10^6 cells/ml, and anti-Fas antibody (10 ng/ml) was added to the medium for 24 h. In some experiments, the broad-spectrum, cell-permeable inhibitor of caspases, ZVAD-fmk (Calbiochem, La Jolla, CA), was added to the culture medium at 50 μM, 90 min before IFA treatment for HT29-C1.16E cells or anti-Fas antibody treatment for Jurkat cells.

Isolation of Human Normal Colonic Epithelial Cells. Histologically normal colonic epithelial cells were isolated from surgical specimens using a nonenzymatic dissociation technique as described previously (19). Preparations of colonic cells were devoid of contamination by immune cells (19).

Immunoblot Analysis. Cells from the various cell lines as well as human colonocytes were lysed for 30 min on ice in RIPA buffer [150 mM NaCl, 0.5% sodium deoxycholate (Sigma), 50 mM Tris-HCl (pH 8.0), 0.1% SDS from Bio-Rad (Ivry/Seine, France)] supplemented with protease inhibitors (1 ml Tris-HCl (R&D System, Oxon, United Kingdom)) for 24 h. Adracyumycin (Sigma Chemical Co., St. Quentin Fallavier, France) was added to the culture medium for the last 12 h of treatment at the concentrations indicated. This treatment with the three agents is referred to as IFA treatment throughout the text. After treatment, both viable adherent cells and floating dead cells were collected.

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RESULTS

ICAD Expression in Human Normal Colonicocytes and Colonic Cancer Cell Lines. We assessed ICAD expression by immunoblotting in both human normal colonicocytes and colonic cancer cells (Fig. 1). ICAD expression was found to be restricted to the functional isofrom of M, 45,000 in both normal colonicocytes (Fig. 1A) and in six tissue culture medium by centrifugation (240 × g for 10 min at 4°C), and the LDH content from the pellets, referred to as LDH<sub>i</sub>, was used as an index of apoptotic cell death (20). The LDH released in the culture medium (extracellular LDH, or LDH<sub>e</sub>) was used as an index of necrotic cell death. The LDH present in the adherent, viable cells was referred to as intracellular LDH (LDH<sub>i</sub>). The percentage of apoptotic and necrotic cell death was calculated as follows: % apoptosis = LDH<sub>i</sub>/LDH<sub>i</sub> + LDH<sub>e</sub> × 100; and % necrosis = LDH<sub>e</sub>/LDH<sub>i</sub> + LDH<sub>e</sub> × 100.

Immunocytochemistry. Cyto centrifuged preparations of HT29-C1.16E cells from six-well plates were fixed for 10 min in cold acetone and incubated with the M30 monoclonal antibody (1:50; Roche Diagnostics) for 1 h at room temperature. This antibody specifically recognizes a cytokeratin 18 neo-epitope that becomes available at an early caspase cleavage event during apoptosis and can be considered as an early marker of apoptosis in epithelial cells (21). The incubation with M30 antibody was followed by a streptavidin-biotin-horseradish peroxidase method using aminobenzyl carbazole as a chromogen (Histostain Plus kit; Zymed, Clincis sciences, Montrouge, France) according to the manufacturer’s instructions. Nuclei were counterstained with hematoxylin. The percentage of cells stained with the M30 antibody was assessed by counting at least 400 cells under the microscope. This technique was also applied to HT29-C1.16E cells cultured on Labtek chambers to ensure that the staining pattern observed on cyto centrifuged preparations was not attributable to the trypsinization process itself.

Hoechst Staining. The DNA-specific dye Hoechst 33258 (Calbiochem), 0.5 μg/ml in HBSS without phenol red (Life Technologies, Inc.) was used on Carnoy-fixed cyto centrifuged preparations of cells. The percentage of apoptotic nuclei was determined by counting under a fluorescence microscope (×400, IX70; Olympus, Rungis, France). At least 600 cells/determination were counted.

Ultrastructural Studies. HT29-C1.16E cells were harvested and centrifuged at 100 × g. The cell pellets were fixed in cacodylate-buffer 3% glutaraldehyde and prepared for electron microscopy by standard methods.

Analysis of DNA Fragmentation by Agarose Gel Electrophoresis. HT29-C1.16E cells were pelleted and suspended in 100 mM NaCl, 10 mM Tris (pH 8.0), 10 mM EDTA, 0.5% SDS, and 0.5 mg/ml proteinase K and incubated for 3 h at 37°C. Then DNA was extracted with phenol:chloroform:isoamyl alcohol (25:24:1), precipitated with isopropanol overnight at −20°C, and centrifuged. DNA was then washed with 70% ethanol and centrifuged, and pellets were dried and suspended in 10 mM Tris/1 mM EDTA. Samples were incubated 30 min at 37°C with 100 μg/ml RNase A (Sigma), and DNA concentrations were determined. Samples were incubated for 20 min at 68°C after addition of loading buffer (Sigma). Ten μg of DNA were analyzed in ethidium bromide-containing 2% agarose precast gel (Invitrogen, Groningen, the Netherlands).

RESULTS

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postconfluent colonic cancer cell lines (Fig. 1, B and C). ICAD-L/DFF45 was expressed independently of the differentiation state of the cells; the clonal derivatives of HT29 cells, HT29-Cl.19A and HT29-Cl.16E cells (13), which differentiate at confluence into ion-transporting polarized cells and typical monolayers of mucus-secreting cells, respectively, expressed ICAD-L/DFF45 at the same level. In addition, the parental undifferentiated HT29 cell line also expressed ICAD-L/DFF45. In contrast, Jurkat cells, used as a control, expressed both the M<sub>b</sub> 15,000 and M<sub>b</sub> 35,000 isofoms (Fig. 1B), as described previously (10, 22). Finally, we examined whether ICAD-L/DFF45 expression was dependent on the growth phase in cultured colonic cancer cells. In preliminary experiments, ICAD-L/DFF45 expression was examined in five cell lines (HT29, HT29-Cl.16E, HT29-Cl.19A, Caco-2, and HGT-1) at two time points, i.e., during the exponential phase of growth (day 4 of culture) and in confluent cells (data not shown). In these conditions, HT29-Cl.16E was the only one to exhibit down-regulation of DFF45 expression during the exponential phase of growth (Fig. 1C). HT29-Cl.16E cells were therefore chosen for additional studies. Time course experiments showed a decreasing expression of ICAD-L/DFF45 from seeding (day 0) up to day 5 of culture (Fig. 1E). Then ICAD-L/DFF45 expression increased over time to reach its maximum at confluence; the fluorescence level of ICAD-L/DFF45 band was 6.4-fold higher at day 14 than at day 5. In contrast, CAD remained essentially stable during the same period of culture (Fig. 1, D and E). These results prompted us to study the consequences of ICAD-L/DFF45 down-regulation on the cellular response of growing HT29-Cl.16E cells after treatment with proapoptotic agents.

**Growth Phase-dependent ICAD-L/DFF45 Expression: Correlation with Nuclear Features of Apoptosis.** Our strategy to examine the consequences of down-regulated ICAD expression was to trigger cell death by using agents, i.e., anti-Fas antibody or Adriamycin, known to activate pathways converging to caspase-3 activation, which is responsible for ICAD cleavage and inactivation (4, 7).

To induce cell death, growing HT29-Cl.16E cells were preincubated for 12 h with anti-Fas antibody (10 ng/ml) plus IFN-γ (10 ng/ml), and then Adriamycin was added to the incubation medium for an additional 12-h incubation (referred to as IFA treatment). The extent of cell death was evaluated by measuring LDH released in the culture medium (as an index of necrotic cell death) and LDH content of floating cells (as an index of apoptotic cell death; see “Materials and Methods”). Adriamycin dose dependently induced both apoptotic (Fig. 2A) and necrotic (Fig. 2B) cell death from growing HT29-Cl.16E cells treated with IFN-γ and anti-Fas antibody. Adriamycin was maximally effective at a concentration of 10 μg/ml. Anti-Fas antibody alone, up to a concentration of 100 ng/ml (data not shown); Adriamycin alone, up to a concentration of 50 μg/ml (Fig. 2B) or the combination of anti-Fas antibody with IFN-γ or Adriamycin (data not shown) were unable to trigger cell death. Several preliminary experiments were performed to verify that cells released in the supernatant upon IFA treatment were actually dead cells; these cells were unable to reaggregate and/or proliferate upon reseeding in fresh culture medium.

To further characterize the IFA-induced apoptotic cell death in growing HT29-Cl.16E cells, it was then important to define additional early and late markers of apoptosis. Cytokeratin 18, expressed in human gut epithelium as well as in a variety of human epithelial cell lines, is a substrate of caspase-3 (23) and was then chosen as an early marker of apoptosis. The cleavage of cytokeratin 18 by caspase-3 generates an epitope that is specifically recognized by the M30 monoclonal antibody (21). As shown in Table 1, most cells released in the supernatant upon IFA treatment (floating cells) scored positive

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**Fig. 1.** Expression of ICAD in human normal colonocytes and cancer cell lines and expression of CAD in HT29-Cl.16E cells. Immunoblot analysis of ICAD expression was performed as described in “Materials and Methods” from five preparations of human normal isolated colonocytes (A), six human colonic cancer cell lines harvested at confluence (B and C), and growing and postconfluent HT29-Cl.16E cells (D). Immunoblot analysis of CAD was performed in growing and postconfluent HT29-Cl.16E cells (D). The Jurkat cell line served as a positive control. Numbers on the left, molecular size of standards in thousands. Quantitative immunoblot analysis of ICAD (●) and CAD (○) expression over time in HT29-Cl.16E cells was performed using enhanced chemiluminescence as described in “Materials and Methods” (E). All levels of ICAD were expressed relative to the lowest expression of ICAD (day 5 of culture), which corresponds to 100%. All levels of CAD were also expressed relative to CAD expression at day 5 (100%). Data points represent one experiment, and another experiment gave essentially the same results.

**Fig. 2.** Induction and characterization of cell death in growing HT29-Cl.16E cells. HT29-Cl.16E cells were treated with increasing concentrations of Adriamycin alone (○) or in combination with 10 ng/ml IFN-γ and 10 ng/ml anti-Fas antibody (IFA treatment, ●). Cell death, assessed by LDH activity-based assays, was expressed as percentage of apoptosis (A) and percentage of necrosis (B), as described in “Materials and Methods.”
with the M30 antibody. We then performed an immunoblot analysis of caspase-3 cleavage during the IFA treatment of growing HT29-C1.16E cells. As shown in Fig. 3A, the IFA treatment led to a typical pattern of caspase-3 activation in floating cells, associated with a loss of procaspase-3. Interestingly, caspase-3 cleavage was also observed to a lesser degree in adherent IFA-treated cells (Fig. 3A), suggesting that the proteolytical activation of caspase-3 was an early event preceding cell detachment. Measurements of caspase-3 activity using a spectrophotometric assay showed that IFA treatment resulted in caspase-3 activation in both adherent and floating cells (29.7 pmol DEVD-pNA/min/10⁶ cells (n = 2) and 35.3 pmol DEVD-pNA/min/10⁶ cells (n = 2), respectively), whereas untreated cells did not display any caspase-3 activity [0 pmol DEVD-pNA/min/10⁶ cells (n = 2)]. This level of activity was in the same order of magnitude as that of anti-Fas antibody-treated Jurkat cells [26.3 pmol DEVD-pNA/min/10⁶ cells (n = 3) versus 0 pmol DEVD-pNA/min/10⁶ cells (n = 2) in untreated Jurkat cells]. Immunoblot analysis of both adherent and floating cells with anti-ICAD antibodies did not disclose any immunoreactivity (Fig. 3B). When confluent HT29-C1.16E cells, used as a control, were treated with IFA, ICAD-L/DFF45 was found to be processed proteo-lytically, as shown by the loss of the M, 45,000 band (Fig. 3D), in parallel with caspase-3 cleavage (Fig. 3C). Altogether, these results show that growing, IFA-treated HT29-C1.16E cells expressed early markers of apoptosis, i.e., retention of cytosolic proteins, cleavage of cytokeratin 18, and proteolytic activation of caspase-3.

We then assessed late markers of apoptosis known to be dependent on ICAD cleavage, i.e., chromatin condensation, nuclear fragmentation, and DNA laddering. Interestingly, Hoechst staining of cytocentrifuged preparations of floating cells from growing, IFA-treated HT29-C1.16E showed nuclei variable in size, with small nuclei exhibiting some degree of chromatin condensation and swollen nuclei without any evidence of chromatin condensation (Fig. 4A). Electron microscopic examination of these cells showed only a peripheral pattern of chromatin condensation with neither chromatin compaction nor nuclear fragmentation (Fig. 4B). Finally, IFA treatment was unable to provoke the typical DNA laddering (Fig. 4E, Lane 2). To understand the mechanisms underlying the IFA-induced cell death of growing HT29-C1.16E cells, we evaluated the cellular responses to the broad-spectrum caspase inhibitor, ZVAD-fmk. This inhibitor (50 μM) was able to inhibit the IFA-induced apoptotic cell death of growing HT29-C1.16E by 65.7% (±2.4; n = 6) without impairment of necrosis (data not shown), based on LDH activity assays. At this concentration, ZVAD-fmk almost fully protected Jurkat cells from apoptosis induced by anti-Fas antibody (92.1% inhibition ± 0.8; n = 3), as demonstrated by Hoechst staining.

In striking contrast, exposure of confluent HT29-C1.16E cells, which express ICAD-L/DFF45, to IFA treatment led to typical apoptotic chromatin condensation and nuclear fragmentation, as shown by Hoechst staining (Fig. 4C) and electron microscopy (Fig. 4D). In addition, these nuclear features of apoptosis were associated with a typical DNA ladder (Fig. 4E, Lane 4). This apoptotic cell death was inhibited by ZVAD-fmk (50 μM; data not shown).

### DISCUSSION

The four main points shown here are: (a) this is the first fully documented report of the ICAD status in human normal colonocytes and in human colon cancer cell lines; (b) ICAD expression was dependent on the growth phase in a homogeneous clonal colon cancer cell line; (c) ICAD down-regulation in proliferating HT29-C1.16E cells was associated with the absence of hallmarks of nuclear apoptosis in response to proapoptotic agents, whereas typical nuclear apoptosis was restored upon treatment of confluent cells; and (d) both forms of death were dependent on caspase activation.

When it came to studying the functional and morphological consequences of ICAD down-regulation, our strategy, based on the HT29-C1.16E model in the exponential phase of growth, was to examine the effects of agents known to trigger the activation of caspase-3. In fact, caspase-3 is responsible for ICAD cleavage and subsequent CAD release, with this nuclease giving rise to the nuclear changes that are the signature of apoptosis. The best characterized apoptotic pathway involving caspase-3 activation and cleavage of its substrate ICAD is the one induced through the surface molecule Fas. The recent demonstration that human colonocytes undergo apoptosis upon Fas ligation (24, 25), together with the present demonstration that human colonocytes express the functional isoform of ICAD, point to a role of ICAD in mediating the apoptotic process triggered by Fas in colonocytes. In contrast, several cancer cell lines are resistant to Fas-induced apoptosis (24, 26). This resistance can be accounted for by down-regulation of Fas receptor or receptor-proximal molecules. In fact, some of these cancer cell lines can be sensitized to the Fas cytotoxic effect by agents, e.g., IFN-γ or Adriamycin, which are known to up-regulate Fas receptor or receptor-proximal molecules (24, 27–33). HT29-C1.16E cells were resistant in our experimental conditions to Fas, and a cytotoxic response could be elicited only upon cell exposure to the combination of IFN-γ, anti-Fas antibody, and Adriamycin (IFA treatment), a finding in line with the concept that these agents trigger synergistic pathways. Indeed, in these conditions, the cytotoxic effect on growing HT29-C1.16E cells was associated with caspase-3 activation and expression of early markers of apoptosis, i.e., intranucleosomal retention of cytosolic proteins (LDH) and cleavage of cytokeratin 18, which is a caspase-3 substrate (23). In contrast, the late events of apoptosis, i.e., intranucleosomal DNA cleavage and nuclear fragmentation, were absent, whereas confluent HT29-C1.16E...
cells, which express ICAD-L/DFF45, exhibited nuclear fragmentation upon IFA treatment. Interestingly, the morphological nuclear features of growing HT29-Cl.16E cells in response to a cytotoxic treatment are remarkably similar to those of murine ICAD knockout (ICAD−/−) and caspase-3 knockout (Caspase-3−/−) oncogenically transformed murine embryonic fibroblasts in response to apoptotic stimuli (11, 34). In addition, the MCF-7 cell line, devoid of caspase-3 because of a 47-bp deletion within exon 3 of the caspase-3 gene, does not undergo typical nuclear changes when treated with tumor necrosis factor or staurosporine (35). Taken together, these findings as well as the present study support the concept that the apoptotic nuclear changes are dependent on the integrity of the caspase-3/ICAD pathway. Because both incomplete apoptotic phenotype displayed by dying proliferating cells and complete apoptotic process displayed by confluent cells were caspase dependent, it is concluded that ICAD inactivation is a dispensable step in caspase-mediated cell death.

During malignant transformation, colonocytes acquire different mechanisms to escape apoptosis mediated by various agents including p53 mutations (36), Fas down-regulation (24), inhibition of Fas capping, or enhanced expression of intestinal trefoil factor (37, 38). DFF45/ICAD down-regulation in proliferative colonic cancer cells could be another mechanism to escape the late changes of apoptosis including nuclear fragmentation and cell disintegration. Here we describe a new phenotypic feature of cell death induced by caspase activation, the biological significance of which remains to be determined in vivo.

In conclusion, this is the first report showing changes associated with ICAD down-regulation in a human colonic cancer cell line. Additional studies are needed to extend this characterization to a large panel of cell lines. ICAD is a potentially important target involved in the control of the apoptotic process in colonic cancer.
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