Clusterin-Mediated Apoptosis Is Regulated by Adenomatous Polyposis Coli and Is p21 Dependent but p53 Independent

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ABSTRACT

Clusterin is a widely expressed glycoprotein that has been paradoxically observed to have both pro- and antiapoptotic functions. Recent reports suggest this apparent dichotomy of function may be related to two different isoforms, one secreted and cytoplasmic, the other nuclear. To clarify the functional role of clusterin in regulating apoptosis, we examined its expression in human colon cancer tissues and in human colon cancer cell lines. We additionally explored its expression and activity using models of adenomatous polyposis coli (APC)- and chemotherapy-induced apoptosis. Clusterin RNA and protein levels were decreased in colon cancer tissues largely devoid of wild-type APC when compared with matched normal tissue controls, suggesting a means for invasive cancers to avoid apoptosis. Conversely, induction of apoptosis by expression of wild-type APC or by treatment with chemotherapy led to increased clusterin RNA and protein levels localizing to apoptotic nuclei. We found that transient transfection of clusterin to colon cancer cell lines directly enhanced basal and chemotherapy-induced apoptosis. Clusterin-induced apoptosis was inhibited by antisense clusterin and was found to be highly dependent on p21 but not p53 expression, yet a deficit in p21 can be subverted by clusterin transfection. Collectively, these data support the hypothesis that nuclear clusterin function is proapoptotic when induced by APC or chemotherapy in the context of p21 expression. Absent of p21, clusterin in not induced, and apoptosis is significantly inhibited. These data support a potential therapeutic role for clusterin in enhancing chemotherapy-induced apoptosis and in promoting apoptosis in cells deficient in p21.

INTRODUCTION

Clusterin is a widely expressed glycoprotein whose function remains somewhat enigmatic. Also known as apolipoprotein J, clusterin was initially characterized as an apoptosis-associated transcript after it was identified as testosterone-repressed prostate message 2 (1) and sulfated glycoprotein 2 (2) that is overexpressed in the epithelial cells of the regressing rat ventral prostate (3). Increases in clusterin mRNA and protein levels have since been consistently detected in apoptotic heart, brain, lung, liver, kidney, pancreas, and retinal tissue both in vivo and in vitro (1, 4–11). Moreover, clusterin-knockout mice show reductions of cell death in hypoxia-ischemia–induced brain damage (12–14). Clusterin function, however, has also been reported to be antiapoptotic and has been found to be overexpressed in progressing breast carcinomas, where its expression correlated inversely with the apoptotic index. Similarly, clusterin-knockout mice show an increase in autoimmune myocardial damage, suggesting an antiapoptotic function.

Recent studies have shed some light on the dichotomous functions of clusterin, suggesting pro- and antiapoptotic functions may be related to nuclear and secreted isoforms, respectively, and that the function of clusterin may be context dependent. Overexpression of the secreted form of clusterin in human cancer cells caused drug resistance and protection against certain cytotoxic agents that induce apoptosis (15). Additional data suggest that a secreted form of clusterin acts as a molecular chaperone, scavenging denatured proteins outside cells following specific stress-induced injury such as heat shock. Other data, show that overexpression of a specific nuclear form of clusterin acts as a prodeath signal (16). Furthermore, studies of human colon cancer suggest a conversion from the nuclear form of clusterin to the cytoplasmic form, which may promote tumor progression (17). Recently, a link between the accumulation of the nuclear form of clusterin and anoikis induction in prostate epithelial cells was shown (18).

To better elucidate the functional role of clusterin, we examined its expression and localization in a panel of normal and neoplastic human colon cancer tissues, as well as a panel of human colon cancer cell lines. We then evaluated the effect of APC expression and chemotherapy, both known to induce apoptosis, on clusterin expression and associated apoptotic events. The effect of clusterin transfection, or alternatively, antisense clusterin transfection, on basal and chemotherapy-induced apoptosis was investigated. Because both p21 and p53 have been linked to regulation of apoptotic events, clusterin expression and induction of apoptosis was evaluated in the context of their expression using targeted knockout cell line models.

MATERIALS AND METHODS

Human Tissues. Snap-frozen human colon cancers (Dukes’ stage C) and their cognate normal mucosal controls were obtained from the Moffitt Cancer Center tissue bank in a de-identified fashion with an Institutional Review Board-approved protocol. Tissues were microdissected for purity and then exposed to cellular lysis buffers and Trizol for RNA extraction.

Cell Lines. Four different human colon cancer cell lines were obtained from the American Type Culture Collection (HT29, KM12C, HCT116, and SW620), and two targeted knockout cell lines were a kind gift of Dr. B. Vogelstein. HT29APC was also a gift of Dr. Bert Vogelstein. HT29, KM12C, HCT116, and SW620 cells were cultured in RPMI medium with 10% fetal bovine serum. HT29APC, HCT116p21+/−/+, HCT116p53+/−+ and their parental cells were cultured in McCoy’s 5A medium with 10% fetal bovine serum. Cells were treated with Zn2+ (12 μmol/L) 4, 8, and 16 hours or with 5-flurouracil (5-FU; 5 μmol/L) and anti-Fas antibody (50 ng/mL) or with irinotecan (6 μmol/L) 24, 48, and 72 hours to induce apoptosis.

Transient Transfection of Clusterin. The plasmids p4 empty vector and p4/SGP2 (containing human clusterin full-length cDNA) were obtained from Dr. Saverio Bettuzzi (Parma, Italy). Transient transfection of p4 empty vector (as a mock) or p4/SGP2 were performed using FuGENE 6 Transfection Reagent (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s protocol. After transfection with mock or clusterin 24 to 48 hours, cells were either harvested or treated by apoptotic stimuli for 48 hours, then harvested to detect clusterin protein expression by Western blotting or to perform apoptosis assays. Each transfection experiment was done in triplicate. Data are presented as a mean ± SE.

Western Blot Analyses. Cells were harvested in protein lysis buffer [20 mmol/L Tris-HCl (pH 7.6), 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5% NP40, 1 mmol/L DTT, 5 μmol/L trichostatin A, 1 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L Na3, and complete protease inhibitors (Roche Applied Science, Indianapolis, IN)], and Western blotting was performed as described previously (19). All blots were performed in...
triplicate. Representative examples are shown in the figures. Antibodies used include clusterin (Alexis, San Diego, CA) and p53 and p21 (BD Biosciences, San Jose, CA).

Antisense Clusterin Oligonucleotide Transfection. Pretreated ~1 × 10^5 HT29, HCT11.6 and HT29APC cells in 100-μm culture dishes, respectively, for overnight. They were transfected with antisense clusterin oligonucleotides or mismatch clusterin oligonucleotides, respectively. Phosphorothioate oligonucleotides used in this study were purchased from Integrated DNA Technologies, Inc. (Corvald, IA). The sequence of the clusterin antisense clusterin oligonucleotide used corresponded to the human clusterin translation initiation site 5′-CAGACGAGGCTTCTACAT-3′. A 2-base clusterin mismatch oligonucleotide (5′-CAGACGAGGATATCTACAT-3′) was used as control. Transfections were performed using the Lipofectamine Plus reagent (Invitrogen, Carlsbad, CA). Cells were treated with either 500 and 1000 nmol/L antisense or mismatch clusterin oligonucleotides after preincubation for 20 minutes with 10 μg/mL lipofectin in serum-free OPTI-MEM (Life Technologies, Inc., Carlsbad, CA). Four hours after the beginning of the incubation, for HT29 and HCT11.6 cells, the medium containing oligonucleotides and lipofectin was replaced with standard culture medium plus 5-FU (5 μmol/L) and Fas (50 ng/mL) once daily for 3 days. For HT29APC cells, the medium was replaced with standard culture medium plus Zn^2+ (120 μmol/L) for 16 hours. Cells were harvested to detect clusterin protein expression by Western blotting or to perform apoptosis assays. Each transfection experiment was done in triplicate and repeated at least three times. Data are presented as a mean ± SE.

cDNA Synthesis and Real-time PCR. To measure clusterin mRNA expression, RNA was isolated from snap-frozen human colon cancers (Dukes’ stage C), and their cognate normal mucosal controls or colon cancer cells treated with different reagents by using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. A quantitative primer/probe set was designed to evaluate and to quantitate clusterin mRNA. Reverse transcription of RNA was performed using 15 units of Omniscript reverse transcriptase (Qiagen, Valencia, CA), 1× cDNA synthesis buffer, 40 units of RNase inhibitor (Life Technologies, Inc.), 5 mmol/L DTT, 1 mmol/L deoxynucleoside triphosphate mixture, 0.5 μg of Oligo(dt) primer, and 2 μg of RNA. Primers and probes for real-time PCR were designed using Primer Express software (Applied Biosystems, Inc., Foster City, CA). Each primer set consisted of standard PCR primers (temperature 58° to 60°C) designed to span gene exons to exclude any possible genomic DNA contamination. Detection and quantitation of each gene was accomplished using an amplex-specific fluorescent oligonucleotide probe (temperature 68°C to 70°C), with a 5′-reporter dye (carboxyfluorescein) and a downstream 3′-quencher dye (6-carboxytetramethylrhodamine). The primers used for detection of clusterin had the following sequences, 5′-CTATCTGCGGGTCACCAC-3′ (forward primer), 5′-CACGTGACCCGGGAAGGAAC-3′ (reverse primer), and 5′-FAM/TGTCCTTCCACACTCTT-GACTCGG/A/TAMRA-3′ (probe). During PCR, the 5′-3′ nuclease activity of TaqDNA polymerase releases the reporter, whose fluorescent signal is then detected by the GeneAmp 5700 Sequence Detector System (ABI, Foster City, CA). The PCR reaction was performed in a 96-well optical reaction plate with optical caps. The reaction mixture consisted of 0.2 μmol/L each primer, 0.1 μmol/L fluorescent probe, 5 units of AmpliTaq Gold (ABI), 200 μmol/L each dATP, dCTP, and dGTP, 400 μmol/L dUTP, 5.5 mmol/L MgCl₂, 8% glycerol, 1 unit of AmplErase uracil-N-glycosylase and 1× TaqMan Buffer A. Amplification profile was as follows: 50°C for 2 minutes (for uracil-N-glycosylase digest of containing PCR amplicons), 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute, 2 μL of cDNA were assayed per well. Standards used for quantitation were plasmid vector DNA containing the primer-specific coding region for clusterin. Standard values, 10⁶, 10⁵, 10⁴, 10³, 10², and 10 DNA molecules per well. Two assays were performed on each cDNA sample, clusterin, and 3-histone H3 as a housekeeping gene to normalize mRNA values. Quantitative PCR was performed in an ABI 5700 sequence detection system.

Apoptosis Assays. To determine apoptotic fractions, cells were harvested at different time point after treatment by apoptotic stimuli. Cells were then analyzed by using the Annexin V-phycocerythrin plus 7-amino-actinomycin D (BD Biosciences) apoptosis detection kit according to the manufacturer’s protocol. The DNA damage experiments were done in triplicate for each cell line. Apoptotic fractions were determined by flow cytometry. Apoptosis was confirmed by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining with the In Situ Cell Death Detection Kit (TMR red; Roche Applied Science) according to the manufacturer’s protocol and immunoblotting with human clusterin antibodies (Alexis).

Immunocytofluorescence and TUNEL Assay. Pretreated ~1 × 10⁶ cells/well were exposed to treatment conditions as planned (some cells were treated 48 hours by apoptotic stimuli, some cells were transiently transfected with clusterin 24 hours, and then treated another 48 hours with apoptotic stimuli). Cells were fixed with acetone-methanol (1:1; Sigma, St. Louis, MO) for 3 minutes, then were blocked with PBS + 10% normal goat serum for 20 minutes. Cells were incubated with clusterin antibody (1:200; Alexis) in PBS + 10% normal goat serum for 2 hours, then the slides were washed with PBS + 0.1% Triton X-100 three times and were incubated with FITC (1:100; Vector Laboratories, Inc., Burlingame, CA) in PBS + 10% normal goat serum for 1 hour and then washed again with PBS + 0.1% Triton X-100. Some slides were dried and covered with coverslips in Vectashield mounting media of anti/face 4′,6-diamidino-2-phenylindole (1:1; Vector Laboratories, Inc.). Immunofluorescence was observed with a Leitz Orthoplan 2 microscope, and images were captured by a charge-coupled device camera with Smart Capture program (Vysis, Downers Grove, IL). Some slides were double staining by TUNEL assay with the In Situ Cell Death Detection Kit (TMR red; Roche Applied Science) according to the manufacturer’s protocol to record clusterin expression and apoptotic cell death co-localization.

RESULTS

Clusterin Is Down-regulated in Human Colon Cancer. To further explore the potential role of clusterin in colon cancer, the levels of clusterin mRNA and protein were assessed in human colon neoplastic specimens (Dukes’ stage C) relative to matched normal mucosal controls from 10 patients. Compared with the matched normal tissues, clusterin mRNA and protein expression were generally down-regulated in human colon cancers (Fig. 1, A and B). Both RNA and protein expression were consistently reduced by >50% in the majority of cases.

Clusterin Is Up-regulated in Chemotherapy-induced Colon Cancer Cell Apoptosis. To determine whether clusterin expression was implicated with colon cancer cell apoptosis, we used two common chemotherapeutical agents for colon cancer, 5-FU (5 μmol/L) and irinotecan (6 μmol/L) for proapoptotic stimuli. Anti-Fas antibody (50 ng/mL) was added to 5-FU because we have previously shown that it significantly enhances apoptosis in colorectal cancer cell lines.
that the combination enhances apoptotic responses in colon cancer cell lines. After treatment, HT29, HCT116, KM12C, but not SW620 had significant increases in apoptosis (Fig. 2A). Apoptosis was assessed by flow cytometric analysis using Annexin V-phycoerythrin. Quantitative PCR and Western blotting were then used to measure clusterin mRNA and protein expression. Clusterin protein expression at baseline was undetectable for all four cell lines and dramatically increased with chemotherapy exposure in all cell lines but SW620 (Fig. 2B). Fluorescence immunostaining and TUNEL staining were used to localize clusterin expression and apoptotic cell death. Clusterin was found to be primarily expressed in the nuclei in the TUNEL-positive stained apoptotic cells after chemotherapy treatment. The results indicated that the fraction of apoptotic cells was increased 5-fold in those cells exposed to apoptotic stimuli relative to controls absent of apoptotic stimuli in HT29 (Fig. 2C) and HCT116 (Fig. 2D), but again, no apoptosis was induced at SW620 colon cancer cell line (data not shown).

Clusterin Is Up-regulated in Wild-type APC-induced Colon Cancer Apoptosis. Because most colon cancers lack functional APC protein and the data suggest diminished clusterin expression in these samples, we turned to a well-described in vitro cell line model in which wild-type APC protein can be induced to cause apoptosis. A null APC−/− human colon cancer cell line (HT-29) stably transfected with a Zn2+-inducible wild-type APC vector or a β-galactosidase control, was used to additionally investigate the relationship between clusterin expression and APC. As demonstrated by real-time quantitative PCR, the clusterin mRNA levels dramatically increased in cells as early as 4 hours after induction of wild-type APC expression (Fig. 2A).}

\[ P < 0.01 \text{ versus untreated control. HT29, HCT116, KM12C, but not SW620 had significant increases in apoptosis with treatment. B, real-time quantitative PCR results showed that clusterin mRNA was up-regulated for all cell lines except SW620, compared with untreated controls. A Western blot analysis showed that clusterin protein expression at baseline was undetectable for all four cell lines and dramatically increased with chemotherapy exposure in all cell lines but SW620. C and D, dual-staining immunofluorescence assay and TUNEL staining stained clusterin antigen (green), apoptotic cell death (red), and nuclei (blue). Clusterin was found to be primarily expressed in the nuclei in the TUNEL-positive stained apoptotic cells after chemotherapy treatment in HT29 and HCT116. DAPI, 4',6-diamidino-2-phenylindole.}\]
induction of wild-type APC expression (Fig. 3A). Similarly, protein levels of clusterin were shown to be up-regulated with induction of wild-type APC expression early after APC induction (Fig. 3B). This induction of clusterin and APC tightly correlate with the induction of apoptosis in these cells (data not shown).

**Antisense Clusterin Oligonucleotide Reduced Chemotherapy- or APC-Induced Apoptosis.** To understand if clusterin expression directly linked to chemotherapy- or APC-induced apoptosis, we used previously described (20) antisense clusterin oligonucleotides to test whether blocking clusterin expression by antisense clusterin oligonucleotide could reduce apoptosis induced by chemotherapy or APC. The results showed that clusterin antisense clusterin oligonucleotides (1 mmol/L), but not mismatch control oligonucleotides, significantly decreased clusterin mRNA and protein expression induced by proapoptotic stimuli (Fig. 4, A and B). Similarly, apoptotic cell death induced by proapoptotic stimuli was decreased by ASO >50%, compared with MM control oligonucleotides in HT29, HCT116, and HT29 APC cells.

**Transient Transfection of Clusterin Localized to the Nucleus and Directly Induced Apoptosis or Enhanced Chemotherapy-Induced Apoptosis in Colon Cancer Cells.** Given the correlation between clusterin overexpression and cell death, we investigated whether clusterin directly contributed to colon cancer cell death. We...
transiently transfected clusterin to the wild-type HT29 cell line. After 24 or 48 hours, clusterin protein expression increased ~10-fold compared with mock-transfected or wild-type cells (Fig. 5A). We used fluorescence immunostaining and TUNEL staining to detect clusterin expression and cell apoptosis principally in nucleus of the transfected cells (Fig. 5B). Flow cytometric analysis also showed that transient transfection of clusterin induced apoptosis in >30% cells, as compared with the mock-transfected or wild-type cells (Fig. 5C). We also found that transiently transfected clusterin enhanced apoptosis ~2-fold in cells treated with 5-FU and Fas as compared with mock-transfected cells treated with 5-FU and Fas (Fig. 5D). These results indicated that transient transfection of clusterin to colon cancer cells either directly induced apoptosis or enhanced chemotherapy sensitivity in human colon cancer cells.

Clusterin Induction in Chemotherapy or APC-Induced Colon Cancer Cell Apoptosis Was p21 Dependent. As a broad-acting cyclin-dependent kinase inhibitor, p21 (WAF1) occupies a central position in the cell cycle regulation of self-renewing tissues. In addition to regulating normal cell cycle progression decisions, p21 integrates genotoxic insults into growth arrest and apoptotic signaling pathways that ultimately determine epithelial cell fate. We have found that chemotherapeutical agents or APC were able to substantially up-regulate p21 expression (Fig. 6A). Because we knew that these treatments also induced clusterin and apoptosis, we investigated clusterin expression in wild-type HCT116p21+/+ and knockout HCT116 p21−/− cells. When HCT116 p21−/− cells were treated with proapoptotic stimuli (5-FU or irinotecan), both clusterin and p21 expression were detected. Absent of p21 expression, however, clusterin was not expressed. Similarly, HCT116p21−/− cells showed significant resistance to 5-FU and Fas-induced apoptosis as compared with the parental cells (HCT116p21+/+; Fig. 6B). These results demonstrated that without p21 expression, clusterin was not induced and apoptosis was inhibited. When clusterin was transiently transfected into HCT116 p21−/− cells, however, clusterin protein was overexpressed and co-localized in the nucleus with apoptotic cells. The percentage of apoptotic cells increased ~6-fold as compared with control cells. Clusterin expression effectively bypassed the requirement for p21 expression, inducing basal levels of apoptosis, and enhancing chemotherapy-induced apoptosis (Fig. 6C). Using the colon cancer cell line SW620, we previously showed its resistance to chemotherapy-induced apoptosis (Fig. 2A) and its absence of clusterin expression (Fig. 2B). Here, we show that the SW620 cell line has no detectable p21 expression nor clusterin expression either with or without chemotherapy treatment (Fig. 6D), potentially explaining why this cell line is resistant to chemotherapy induced apoptosis. Taken together, our results suggest that clusterin acts downstream of p21 and that clusterin overexpression in chemotherapeutical-induced apoptosis is p21 dependent.

Clusterin and p21 Induction in Chemotherapy-Induced Colon Cancer Cell Apoptosis Are p53 Independent. The tumor suppressor gene p53 is the primary transcription factor for the induction of p21. To determine whether p53 expression is implicated to p21-clusterin signal transduction pathway, we evaluated p53 expression in HT29 (p53 mutant) and HCT116 (p53 wild-type) colon cancer cell lines. We found that p21 and clusterin were overexpressed, but p53 levels were unaffected in cells after chemotherapeutic treatment (Fig. 7A). We also detected p21 and clusterin expression in HCT116 p53−/− cells after treatment with chemotherapy (Fig. 7B). The results suggest that clusterin and p21 up-regulation by chemotherapeutical treatment is independent of p53 expression.

DISCUSSION

Although clusterin was originally proposed to be a marker for programmed cell death, it has variously been proposed to provide both pro- and antiapoptotic functions. Studies of clusterin-transfected renal carcinoma cells suggest that clusterin expression may increase cell survival, motility, invasive capacity, and contribute to metastasis (14). In Apc Min models of colon neoplasia, tumor cells undergoing apoptosis expressed low levels of clusterin (21). In cancer patients, overexpression of clusterin has been linked to tumor progression in both breast and colon cancer (17, 22). These data support an antiapoptotic role for clusterin. Conversely, a number of studies also support a
proapoptotic role for this molecule. Previous studies suggest that clusterin expression decreases the proliferation rate of prostate epithelial cells (23) and is reduced in human prostate cancer (24), consistent with a proapoptotic function. In addition, knockout studies show reductions in cell death under stress conditions (14, 25).

Our investigations sought to clarify the functional role of clusterin in human colon cancer. Only recently has it come to light that two forms of clusterin, a secreted and a nuclear form, may be responsible for this divergent behavior. It is now apparent that the endogenous nuclear clusterin is a product of alternative splicing. The shorter
is induced in cells undergoing apoptosis stimulated by chemotherapy exposure. Because APC is mutated in the vast majority (>95%) of sporadic colon cancers and APC expression has been linked to the induction of apoptotic events (19), we investigated the role of clusterin in APC-induced apoptosis. We identified that apoptosis induced by APC in APC-null colon cancer cells or by chemotherapy was linked to significant increases in clusterin expression. These data suggested that it was the nuclear form of clusterin (proapoptotic form) that contributes to human colon cancer apoptosis by colocalizing clusterin to apoptotic nuclei. In addition, we found that the stress induced by chemotherapies readily elevated clusterin levels and produced apoptosis.

Because clusterin has been proposed to regulate the cell cycle, increasing the accumulation of cells in the G_0-G_1 phases of the cell cycle (14), we elected to evaluate the potential relationship between p21 and p53 and clusterin expression. The finding that targeted inactivation of p21 enhances Apc-initiated tumor formation (26) also led us to study this relationship further. Our data suggest that clusterin is induced in cells undergoing apoptosis stimulated by APC expression or by chemotherapy exposure. Moreover, we found a coordinate up-regulation of p21 and clusterin expression in cells undergoing apoptosis in response to these stimuli. The question then arose as to whether p21 expression was necessary for clusterin expression. To examine this question, we evaluated human colon cancer cells with a targeted deletion of p21 (HCT116p21^{-/-}) and found that when treated with proapoptotic stimuli, no clusterin overexpression was seen and significantly less apoptosis was induced. Interestingly, the only cell line that was not susceptible to chemotherapy-induced apoptosis, SW620, was also incapable of mounting a p21 response, presumably preventing clusterin expression. Furthermore, when clusterin was transiently transfected into HCT116 p21^{-/-} cells, clusterin protein was overexpressed in the nucleus and colocalized with TUNEL staining in the apoptotic cells. The percentage of apoptotic cells increased ~6-fold as compared with control cells, suggesting that clusterin can effectively bypass the action of p21 and induce apoptosis in the absence of p21. We also found that p21 and clusterin were overexpressed, but p53 levels were unaffected in cells after chemotherapeutic treatment. Similarly, p21 and clusterin were overexpressed in HCT116 p53^{-/-} cells after stimulation by chemotherapy (Fig. 6, I and J). Taken together, these results suggest that clusterin acts downstream of p21 and that clusterin overexpression in chemotherapy-induced apoptosis was p21 dependent and p53 independent. This work is the first to demonstrate a novel link between the nuclear form of clusterin overexpression and the induction of p21.

The p21 signaling pathway that regulates the nuclear form of clusterin expression after chemotherapy exposure is unknown. Induction of the cyclin-dependent kinase inhibitor p21 is a common mechanism of growth arrest in different physiologic situations. Ectopic overexpression of p21 leads to cell growth arrest in G_{1} and G_{2}, possibly by inhibition of cyclinB1/cyclin-dependent kinase 1 as demonstrated in prostate cancer cells. This arrest is accompanied by apoptotic cell death. Although p21 is not a transcription factor, it is conceivable that indirect effects of p21 on cellular gene expression may mediate some of its functions. For example, transient transfection assays showed that p21 could stimulate nuclear factor-κB-mediated transcription; this effect of p21 has been explained through the interaction of cyclin-dependent kinase 2 with transcriptional cofactor p300 that augments nuclear factor-κB and other inducible transcription factors. p21 interactions with proteins other than CDK may also have a potential effect on gene expression. For example, p21 was reported to bind c-Jun NH_{2}-terminal kinases, apoptosis signal-regulating kinase 1, and Gadd45 (27). It was already shown that clusterin promoter region has AP-1 protein (c-Jun and c-Fos) binding sites. In a study by Jin et al. (28), transforming growth factor-β induced clusterin expression in a variety of cell types via a consensus AP-1 binding site.

Collectively, the presented data demonstrate that overexpression of the nuclear form of clusterin either enhanced chemotherapeutic sensitivity or directly induced colon cancer apoptosis. The induction of clusterin by chemotheraphy was p21 dependent but p53 independent. Because clusterin transfectionverted the absence of p21 and caused apoptosis, clusterin protein appears to be an important protein for regulating chemotherapy-induced apoptosis and may have a potential therapeutic role.

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