Downregulation of p63 upon exposure to bile salts and acid in normal and cancer esophageal cells in culture

Sabine Roman,1,2,3,4 Aurélie Pétré,1 Amélie Thépot,1 Agnès Hautefeuille,1 Jean-Yves Scoazec,3,4 François Mion,2,3,4 and Pierre Hainaut1

1Group of Molecular Carcinogenesis and Biomarkers, International Agency for Research on Cancer, Lyon; 2Hospices de Lyon, Digestive Physiology Department, Lyon; 3Claude Bernard University, Lyon I; and 4Institut National de la Santé et de la Recherche Médicale Unité 45, Lyon, France

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Barrett’s esophagus

BARRETT’S MUCOSA is defined as the replacement of the stratified esophageal squamous epithelium by a metaplastic epithelium containing goblet cells (8). This complication of gastroesophageal reflux disease (GERD) is a premalignant condition that predisposes to esophageal adenocarcinoma, a tumor with an increasing frequency in many Western countries (15). Patients with Barrett’s esophagus have an increased bile salt and acid exposure in the lower esophagus in comparison with controls and patients with uncomplicated GERD (26). It has been suggested that bile salt and acid could perturb the differentiation of squamous esophageal cells and promote their reorientation to the glandular differentiation pathway (12). However, the molecular mechanisms responsible for this effect are not known. Understanding these mechanisms and their regulation is essential for the development of successful chemoprevention strategies for the formation of Barrett’s mucosa and its transformation into adenocarcinoma.

Barrett’s esophagus is a specialized intestinal mucosa containing goblet cells, endoscopically detectable as a salmon pink mucosal segment of irregular shape, contiguous to the esophagogastric junction (11). This mucosa is characterized by a pattern of cytokeratin (CK) expression that combines CK20, a common marker of intestinal differentiation, and CK7, which is considered as a marker of ductal differentiation. In addition, expression of CK13, a marker of squamous mucosa, is lost in Barrett’s esophagus (21). Barrett’s esophagus has high proliferative indexes associated with altered expression of multiple growth factors, of inducible nitric oxide synthase, and of cyclooxygenase-2 (COX-2; see Ref. 17). The mechanisms of Barrett’s esophagus formation are still a matter of conjecture. It has been proposed that, under exposure to gastric reflux, squamous mucosa or associated glandular esophageal ducts may undergo altered differentiation, producing both microvilli and intercellular ridges, resulting in a unique glandular phenotype distinct from adjacent mucosal gastric cells (12). However, the mechanistic basis of the reprogramming of squamous and/or duct cells into cells with glandular features remains to be identified.

The TP63 gene encodes six different protein isoforms with homology to the tumor suppressor protein p53. The strongest homology lies in the DNA-binding domain, which is common to all isoforms (4). The isoforms differ in their NH2-terminal and/or COOH-terminal domains. In the NH2 terminus, two types of isoforms can be distinguished, transcriptionally active (TA), which contains the NH2-terminal activation domain, and N, which lacks the TA domain because of usage of an alternative promoter located in intron 3. In the COOH terminus, alternative splicing generates three distinct variants of different length (α, β, and γ; see Ref. 1). Although the exact role of each isoforms is ill-defined, there is evidence that spatial and temporal compartmentalization of the expression of TA and βN isoforms is essential for keratinocyte stem cell differentiation and proliferation (1). Specifically, βN p63 isoforms are strongly expressed in proliferative stem cells and keratinocytes, whereas expression of TA isoforms occurs in suprabasal cells during the commitment to terminal differentiation (6). Mice lacking TP63 (p63−/− mice) show a number of defects in epithelial and mesenchymal differentiation and morphogenesis. In particular, they lack normal skin devel-
opment because of the inability of skin keratinocytes to establish a stable, stratified epithelium. Of note, the esophageal lining of these mice shows a pseudostratified columnar appearance with absence of goblet cells (28), similar to Barrett’s esophagus. This observation, in conjunction with the fact that p63 is expressed in the basal and suprabasal layers of human esophageal squamous epithelium but not in Barrett’s esophagus (10), leads us to suggest that downregulation of p63 expression may represent a critical molecular event in Barrett’s esophagus formation.

In this study, we have exposed squamous esophageal cells, either primary cultures or squamous cell carcinoma cell lines, to treatments with bile salts and acid that mimic the effects of gastric reflux. We show that these treatments induce the rapid downregulation of p63 proteins through a mechanism partially blocked by proteasome inhibitors. These results show that components of gastric refluxes activate a pathway that destabilizes p63, providing a plausible mechanism for the reprogramming of squamous esophageal stem cells into intestinal-type cells during the early steps of Barrett’s esophagus formation.

MATERIALS AND METHODS

Cell cultures and treatments. The esophageal squamous carcinoma cell lines TE-1 and TE-13 (3, 19) were used. TE-cells were grown in RPMI 1640 medium (GIBCO) supplemented with 10% heated FBS, 1% antibiotics (penicillin, streptomycin), and 2 mM L-glutamine. Medium was acidified with 1 N HCl, a pH lower than the pKa for DCA at pH 5. At the end of exposure, cells were harvested by trypsinization, and 2,000 cells were cultured in 60-mm plates in RPMI 1640 medium (GIBCO) supplemented with 10% heated FBS, 1% antibiotics (penicillin, streptomycin), and 2 mM L-glutamine. Medium was changed every 3 days. After 10 days, plates were stained with Giemsa.

Trypan blue assay. TE cells were cultured in six-well plates (400,000 cells/well). After 5 to 80 min exposure to 50 μM DCA at pH 5, cells were harvested with trypsin and counted after trypan blue staining. The results are expressed as a percent increase in the number of dead cells over control. A mean of nine experiments per condition was calculated and compared with control using paired t-test. A difference was considered as significant if P < 0.05.

Flow cytometry. Cell cycle was analyzed by flow cytometry using FACsCalibur (Becton-Dickinson, Mountain View, CA) and a Cycle TEST PLUS staining kit (Becton-Dickinson) according to the manufacturer’s instructions. Cell cycle analysis was repeated in four separate experiments. The percentage of sub-G1 cells, indicative of apoptosis, was expressed as means (+SD) and compared by paired t-test with the percentage of apoptotic cells before DCA (50 μM, pH 5) exposure. A difference was considered as significant if P < 0.05.

Total protein extraction and Western blot analysis. Cells were lysed by adding lysis buffer [50 mM Tris·HCl, pH 7.4, 250 mM NaCl, 0.1% SDS, 0.5% Nonidet P-40 (NP-40), and protease inhibitors (2 μg/ml aprotinin, 500 μM phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 1 pg/ml pepstatin and 2 mM dithiothreitol)] and placed on ice for 30 min. Extracts were subsequently centrifuged at 13,000 rpm for 10 min. The supernatant was taken for Western blot analysis. The cell membrane was extracted by 20 g/ml saponin, and then washed three times with PBS for 10 min.

Lactacystin (20 μM; Sigma) or 50 μM MG-132 (Calbiochem) were added to the culture medium 6 h before cell exposure to DCA and acid exposure to inhibit proteasome activity. Measurements of cyclin A levels were used as a control of the efficiency of proteasome inhibition.

Fig. 1. Profiles of p63 isoform expression. Lanes 1–6: cloned cDNA corresponding to different p63 isoforms transfected in Hep 3B and analyzed by Western Blot; lane 7: TE-13 cells; lanes 8 and 9: TE-1 cells at 37 and 32°C, respectively. Different antibodies against p63 were used as follows: 4A4, which recognizes all p63 isoforms; anti-TAp63, which recognizes transcriptionally active (TA) isoforms; anti-ΔNp63, which recognizes ΔN isoforms; and anti-p63α, which recognizes p63 α isoforms. Arrows on right indicate the expected position of the major p63 isoforms. ΔN isoform appears to be the predominant p63 isoform in TE-1 at 32°C, at 37°C, and in TE-13.
for 15 min at 4°C. Supernatants were then collected. Protein concentration was determined using the method reported by Bradford (5).

Whole protein extracts were loaded, separated by SDS-PAGE with the use of a 7.5%, gel and transferred to polyvinylidene difluoride membranes by electroblotting. Membranes were blocked with 5% dry milk in PBS-NP-40 (0.05%) for 1 h at room temperature and incubated overnight with primary antibody diluted in PBS-NP-40 containing 1% dry milk. The following antibodies were used: mouse monoclonal anti-p63 at 1:500 (4A4, Ab1; Oncogene), which recognizes all p63 isoforms; rabbit polyclonal anti-TAp63 at 1:500 (Biolegend), which recognizes TAp63 isoforms; rabbit polyclonal anti-ΔNp63 at 1:1,000 (anti-P40; Calbiochem), which recognizes ΔNp63 isoforms; rabbit polyclonal anti-αNp63 at 1:500 (H129; Santa Cruz), which recognizes p63 α-isofoms; mouse monoclonal anti-p53 at 1:3,000 (DO7; Dako); goat polyclonal anti-COX 2 at 1:1,000 (C-20; Santa Cruz); mouse monoclonal anti-CK13 at 1:500 (Chemicon); mouse monoclonal anti-CK13 at 1:500 (Chemicon); rabbit polyclonal anti-cyclin A at 1:1,000 (H-432; Santa Cruz); mouse monoclonal anti-actin at 1:20,000 (C-4; ICN Biomedicals); or rabbit polyclonal anti-Ku80 at 1:40,000 (Serotec). The membranes were washed and incubated with peroxidase-conjugated secondary antibody, either goat anti-mouse at 1:10,000 (Dako), donkey anti-goat at 1:5,000 (Santa Cruz), or goat anti-rabbit at 1:3,000 (Dako). Proteins were detected by chemiluminescence (Amersham Biosciences). Actin and Ku80 were used as protein loading controls.

RNA extraction and RT-PCR. Total mRNA from cells was extracted by using Tri-Zol Reagent (Invitrogen), and first-strand cDNA
was synthesized from total RNA using random hexamer primers and the SuperScript II system for RT-PCR (Invitrogen). TAP63 cDNA (forward 5'-GACCTGAGTgACCCCATGTG-3' and reverse 5'-CGGTTGGATGGAGAGAGCA-3') and ΔNP63 cDNA (forward 5'-GACCTGAGTgACCCCATGTG-3' and reverse 5'-AGGAGAGCACTCAGAGTG-3') were coamplified with H9252-actin (forward 5'-GACACCACTGGAGGGTGACT-3' and reverse 5'-CCACATGTTCTCCTGTT-3') for semiquantitative analysis. PCR was performed for 25 cycles for H9252-actin, for 28 cycles for H9004 Np63, and for 30 cycles for TAp63. Amplified products were analyzed on 2% agarose gels. Real-time PCR was also performed with ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Courtaboeuf, France). Relative quantification was performed in a multiplex reaction using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping, control gene (24). The primers and fluorogenic probes for PCR reaction were the following: TAp63 forward 5'-CACACAGACAAATGAATTCCTCAGT-3', reverse 5'-CATCACCACACACTGGAGTCTTGTCTCCTGTC-3' and 6-FAM-GGGATTTTCTGGGAACAGCMGB and ΔNP63 forward 5'-GGAAAACCATGCACACTGGAGTCTTGTCTCCTGTC-3', reverse 5'-TGTTGCAGGAGCCACAGTT-3'/5'-6-FAM-TTAGGCCCCAGTACCTGAGTCT-3', and 6-FAM-TTAGGCCCCAGTACCTGAGTCT-3'. Each sample was analyzed in duplicate, and each experiment was done in triplicate. Relative quantification was performed using the comparative threshold cycle (CT) method as described in the User Bulletin, ABI Prism 7900 HT Sequence Detection System. The relative expression level of the TP63 gene in the different conditions was estimated by calculating the ΔCT value, defined as the difference in the CT value for the target (TAP63 or ΔNP63) and the reference gene (GAPDH; see Ref. 9). Experiments were done in triplicate. Results were expressed as the mean of ΔCT (+SD) and compared with paired t-test. A difference was considered as significant if P < 0.05.

Detection of Intracellular Localization of p63 by Immunofluorescence

Immunofluorescence was performed on cells grown on glass slides and fixed in 4% formaldehyde for 30 min at room temperature. After washing with PBS, cells were permeabilized with 1% Triton X-100 for 10 min and then washed with PBS. After being blocked with 1% BSA in PBS for 1 h, cells were washed with PBS and incubated with rabbit polyclonal anti-p63 antibody (H137; which recognizes all p63 isoforms; Santa Cruz) at room temperature for 1.5 h. After being washed with PBS three times, cells were incubated with a secondary antibody (FITC-labeled anti-rabbit immunoglobulins) at room temperature for 1 h. After being washed with PBS three times, cells were incubated with a secondary antibody (FITC-labeled anti-rabbit immunoglobulins) at room temperature for 1 h. After being washed with PBS three times, cells were incubated with a secondary antibody (FITC-labeled anti-rabbit immunoglobulins) at room temperature for 1 h. After being washed with PBS three times, cells were incubated with a secondary antibody (FITC-labeled anti-rabbit immunoglobulins) at room temperature for 1 h.
RESULTS

Identification of p63 isoforms expressed in TE cells. The esophageal squamous carcinoma cell lines TE-1 and TE-13 (19) both express various p63 isoforms. Figure 1 shows the profiles of p63 isoform expressions using different antibodies. As a reference to identifying isoforms, cloned cDNA corresponding to each isoform were transfected in Hep 3B, a hepatocellular carcinoma cell line that expresses low levels of p63, and analyzed by Western Blot. The TE-1 expresses a temperature-sensitive mutant p53, V272M, that adopts a wild-type conformation at 32°C and a mutant conformation with loss of DNA binding at 37°C (16). We have used this cell line to assess the effect of exposure to acid and bile in cells expressing either active (wild-type) or inactive (mutant) p53 protein. The temperature shift does not significantly alter the expression of p63 (Fig. 1). TE-13 lacks p53 protein expression because of homozygous mutation at the donor splice site of exon 5 and abnormal splicing (3). In both cell lines, the most abundant p63 isoform is ΔNp63α (70 kDa; Fig. 1). Faint bands may correspond to ΔNp63β and TAp63γ isoforms.

Synergistic effect of bile salts and acid exposure on p63 and p53 expression in TE cells and in primary epithelial esophageal cells. First, we have exposed esophageal squamous cells to bile salts and acid, either separately or together, and we have examined their effects on the expression of p63. In TE-13 cells, p63 decreased drastically after 24 h exposure to DCA and acid, whereas acid alone had no effect and DCA alone only slightly decreased p63 expression (Fig. 2A). Similar results were observed in TE-1 cells at 32°C and 37°C after a 1-h pulse of DCA and/or acid (Fig. 2B). In these cells, a p53 decrease in p53 levels was also observed upon DCA and acid exposure. Next, we compared the changes in p63 levels with those of COX-2, an enzyme typically induced by reflux stress and expressed at high levels in Barrett’s esophagus (13). The decrease in p63 levels was associated with an increase in COX-2 protein levels in TE-cells exposed to a 1-h pulse of DCA. DCA increased COX-2 expression both at pH 5 and pH 7. COX-2 expression was also slightly increased at pH 5 alone. The same results were observed in TE-13 exposed for 24 h continuously or for a 10-min pulse, as well as after a 1-h pulse in TE-13 and in TE-1 at both 32°C and 37°C (Fig. 2, A and B).

A decrease in p63 levels was also observed in monolayer cultures of primary esophageal epithelial cells (Fig. 3). In contrast with squamous cells, treatments with acid and bile increased p53 protein levels in Bic-1 and had only small effects at higher doses in Seg-1, two cell lines derived from Barrett’s adenocarcinoma (Fig. 3). The former expresses a mutant p53 protein, whereas the latter expresses wild-type p53, explaining

Fig. 7. DCA and acid induce a moderate cytotoxicity in TE cells. A: cell cycle was analyzed by flow cytometry in TE-1 cells at 37°C after labeling with propidium iodide. No difference in cell cycle distribution was observed before and after 20 min exposure to 50 µM DCA at pH 5. B: percentage of apoptotic cells detected by flow cytometry (mean ± SD) in TE-1 cells exposed for 5–80 min to 50 µM DCA at pH 5. The percentage of sub-G1 cells did not differ significantly before and after treatment (paired t-test, P > 0.05). C: cell viability was assessed in TE cells after 5–80 min of exposure to DCA and acid using trypan blue exclusion. The results were expressed as a percent increase in the number of dead cells over control. A mean of 9 experiments/condition was calculated and compared with control using paired t-test. The percentage of dead cells is significantly higher in all conditions in comparison with untreated cells (P < 0.05) but did not differ with time of DCA and acid exposure (5–80 min).
the variations in p53 levels between the two lines. These two cell lines did not express detectable levels of p63 proteins.

To assess whether the decrease in p63 protein levels was associated with a decrease in other markers of squamous cell differentiation, we analyzed the expression of CK13 after exposure of TE-1 cells at 32°C or 37°C to DCA and acid for 1 h. CK13 is expressed in squamous mucosa but not in glandular mucosa. Figure 4 shows that this treatment induces a small but consistent decrease in the levels of CK13, suggesting that the rapid decrease in p63 levels does not result from a general downregulation of all markers of squamous differentiation.

Compatible results were obtained with CDCA. However, exposure to the taurine conjugate, TCDCA, did not induce a decrease in p63 levels in TE cells (Fig. 5). Together, these results indicate that combined treatment with unconjugated bile salts and acid induces a spectacular downregulation of p63 protein, occurring in the same time frame as the increase in COX-2 expression, which is characteristic of esophageal squamous cells exposed to reflux stress.

Mechanisms of DCA- and acid-induced p63 downregulation. To address the mechanism of the observed decrease in p63 levels, we first sought to determine whether this decrease was occurring in a context of general cytotoxicity. In Fig. 6, we show that p63 and p53 protein levels significantly decreased after 10 or 20 min of exposure to DCA and acid. Flow cytometry analysis within this time frame did not reveal substantial changes in cell-cycle distribution nor an increase in sub-G1 apoptotic cells, as shown in TE-1 at 37°C (Fig. 7, A and B). Using trypan blue exclusion, cell viability was found to be preserved for up to 80 min of treatment, despite a small increase in the percentage of dead cells (Fig. 7C). Finally, Western blot analysis did not reveal an increase in the cleavage of poly-ADP-ribose-polymerase, a substrate of caspase 3 that is an early marker of apoptosis (data not shown). Thus the decrease in p63 observed within 10–20 min of exposure to acid and bile salts cannot be ascribed to generalized cell death. Further assessment of cell survival using a clonogenic assay was carried out after treatment of cells with bile salts and acids for up to 80 min, followed by long-term culture in fresh medium. These experiments showed that cells kept the ability to clonally proliferate even after 80 min of exposure (data not shown).

Fig. 8. Effect of doxorubicine on p63, p53, and COX-2 protein level in TE cells. p53, p63, and COX-2 expression was studied by Western blot after 24 h exposure to different doses of doxorubicin (250, 500, and 1,000 ng/ml). The following antibodies were used: 4A4, which recognizes all p63 isoforms; DO7, which recognizes p53; and C-20, which recognizes COX-2. Actin was used as a control of protein loading.

Fig. 9. TA and ΔΔp63 transcripts after DCA exposure. A: qualitative PCR of p63 mRNA in TE-1 cells at 37°C after 5, 10, and 20 min exposure to 50 μM DCA at pH 5. The expression of TA and ΔΔp63 isoforms is shown on RT-PCR gel. Actin is used as a PCR control. B: real-time PCR in TE cells exposed for 20 min to 50 μM DCA at pH 5. Relative expression levels of TA and ΔΔp63 in comparison with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), expressed as Δthreshold cycle (CT) values, are not significantly modified after 20 min exposure of DCA at pH 5 (paired t-test, P > 0.05).
To determine whether the reduction in p63 levels was a common response to genotoxic stress damage, we analyzed the effects of treatment with doxorubicin, an inhibitor of topoisomerase II that induces single-strand DNA breaks. Figure 8 shows that this treatment did not induce the same extent of p63 downregulation as bile salts and acid. However, a small, dose-dependent decrease in p63 was detected, resulting in a small reduction of the p63 signal detected by Western blot after treatment with doxorubicin at 1 μg/ml.

Next, we analyzed p63 mRNA levels within minutes of exposure to determine whether the decrease in protein levels was preceded by a similar decrease in mRNA levels. We found that p63 mRNA levels were not significantly reduced by DCA and acid exposure (Fig. 9A). Real-time, quantitative PCR showed that DCA and acid exposure did not significantly modify the respective levels of TA and ΔNp63 transcripts (Fig. 9B). Thus the decrease in p63 protein levels does not appear to result from a shut down of transcription.

To determine whether the apparent drop in p63 levels may be because of the relocalization of the protein in a different cell compartment, the localization of p63 after DCA and acid exposure was analyzed by immunofluorescence. At baseline, p63 was expressed in the nucleus of the cells. After 20 min of DCA and acid exposure, p63 staining was clearly decreased in the nucleus and slightly increased in the cytoplasm, indicating that the protein may undergo a drift from nucleus to cytoplasm (Fig. 10). Finally, we used proteasome inhibitors to determine whether the decrease in p63 protein may be the result of active protein degradation by the proteasome. We found that MG-132 prevented the decrease in p53 and p63 levels induced by DCA and acid (Fig. 11). Similar results were observed when proteasome was inhibited by lactacystin (data not shown).

**DISCUSSION**

The mechanisms that lead to the formation of Barrett’s esophagus imply the reprogramming of differentiation patterns of the esophageal mucosa under stress induced by reflux. This process is likely to involve at least two steps, with first the reorientation of differentiation from squamous to glandular patterns and second the selection and survival of the latter cell type as the fittest to survive in a reflux environment. In recent years, compelling evidence has accumulated that the p63 protein is an essential regulator of squamous differentiation. In the present study, we have tested whether in vitro treatments mimicking reflux conditions may affect the status of p63 in esophageal squamous cells. Our results clearly show that bile and acid exposure can downregulate both p53 and p63 protein levels in esophageal cell lines as well as in primary esophageal cell cultures, in conditions in which a characteristic marker of stress and inflammatory responses, COX-2, is induced. This observation suggests that downregulation of p63 may be a critical adaptive response of squamous cells to stress by reflux.

The p63 protein and its various isoforms play a critical role in the development and maintenance of stratified epithelial tissues (6). Using a loss of function approach, Barbieri and colleagues (2) showed that disruption of p63 expression in squamous cell lines and keratinocytes led to downregulation of markers of squamous epithelium and that keratinocytes with reduced p63 expression failed to properly differentiate in vitro. This observation is compatible with the notion that p63 is indeed required for squamous differentiation. In agreement with this notion, we have observed that the decrease in p63...
induced by treatment with bile salts and acid was followed by a small but consistent reduction in the expression of CK13, a typical marker of squamous differentiation. Thus the rapid downregulation of p63 by bile and acid may represent a very early event in the sequence of events leading from squamous esophageal epithelium to Barrett’s metaplasia.

In humans, it has been shown that the toxicity of bile acids toward the esophageal mucosa is dependent on pH (18). In vitro studies have suggested that bile and acid have synergistic effects (25). In concordance with this idea, we have observed a synergistic effect of unconjugated primary or secondary bile acids (CDCA and DCA, respectively) and pH 5 in inducing a decrease in p53 and p63 protein levels. The biological properties of bile salt are strongly pH dependent. Bile acids are partly in an unionized state and are soluble at pH below pKa of bile salt. Only unionized forms of bile acid can penetrate through cell membranes. In contrast, unconjugated bile acids have a pKa around 6 (7). Thus, at pH 5, DCA and CDCA are capable of penetrating through cell membranes and exerting their effects. On the contrary, TCDDA, insoluble at pH 5 (pKa = 2), had no effect on p63 and p53 expression, suggesting that bile salts have to penetrate into cells to modify p63 expression.

DCA and acid exposure induced COX-2 expression in esophageal cell lines. This result is consistent with previous results (14, 22). This observation indicates that the decrease in p63 levels occurs in the same general signaling context as the one that is responsible for the increase of COX-2 protein levels. Whether this result is because of a role of p63 as an inhibitor of COX-2 expression or because of the fact that decreasing p63 levels in squamous cells induces an overall cellular stress situation remains to be investigated.

This study demonstrates that p63 protein isoforms may undergo regulation by proteasome-dependent degradation in response to stress by bile and acid. Regulation of p63 by degradation has also been reported in response to genotoxic response to stress by bile and acid. Regulation of p63 by proteasome-dependent degradation in response to stress by bile and acid. Interestingly, this degradation pathway appears to be cell-type specific, since DCA treatment did not induce a similar drop in p53 levels in two esophageal cell lines derived from Barrett’s adenocarcinoma, Bic-1 and Seg-1.

In conclusion, we show here that DCA at pH 5 decreased p63 protein levels by inducing its degradation by the proteasome and increased COX-2 protein levels in esophageal cell lines and primary esophageal cell cultures. These observations are consistent with the hypothesis that specific downregulation of p63 by bile and acid may represent a primary event in response to reflux stress. Loss of p63 in squamous cells may prevent adequate cicatrisation of ulcerated areas of the esophageal mucosa, leading to the replacement of squamous by glandular cells, a type of cell that is more resistant to reflux stress. It is also possible that stress-induced degradation of p63 facilitates the differentiation of surviving stem cells into an glandular differentiation pathway.

REFERENCES


