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GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells

Celecoxib, a selective cyclooxygenase-2 inhibitor, is known to possess anti-inflammatory activity and also induces apoptosis in various types of cancer cells. Here, we examined the molecular mechanism of celecoxib-induced apoptosis in cervical cancer cell lines (HeLa, CaSki and C33A). Screening of a cDNA microarray chip containing 225 different genes revealed that GADD153 (growth arrest and DNA damage inducible gene), a transcription factor involved in apoptosis, showed the strongest differential expression following celecoxib treatment in all three cervical cancer cell lines. Notably, siRNA-induced silencing of GADD153 suppressed celecoxib-induced apoptosis in all three cell lines, and exogenous expression of GADD153 triggered apoptosis in cervical cancer cells in the absence of other apoptotic stimuli. A luciferase reporter gene assay and mRNA stability tests revealed that the expression of GADD153 was regulated at both the transcriptional and post-transcriptional levels following celecoxib treatment. The region between −649 and −249, containing an intact C/EBP-ATF binding site, is required for celecoxib-induced stimulation of GADD153 promoter activity. In terms of signaling pathway, addition of the NF-κB inhibitor, N-tosyl-L-phenylalanyl-chloromethyl ketone, had no effect on GADD153 expression levels. Celecoxib treatment induced Bak expression, whereas cell transfected with siGADD153 showed lower levels of celecoxib-induced Bak upregulation. These novel findings collectively suggest that GADD153 may play a key role in celecoxib-induced apoptosis in cervical cancer cells by regulating the expression of proapoptotic proteins such as Bak.

Introduction

Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor commonly used to treat chronic arthritic conditions, has recently been shown to induce apoptosis in various types of cancer cells, including colon, prostate, head and neck and cervical cancers (1–5). Several ongoing clinical trials have been undertaken to evaluate the use of celecoxib alone or in combination with other agents for the prevention or treatment in lung cancer (6). Thus, it is important that we understand the anticancer mechanism of celecoxib with the goal of enhancing its efficacy as valuable adjunct or single agent in anticancer therapy.

Celecoxib can induce apoptosis even in COX-2 negative cells, suggesting the presence of a COX-2 independent mechanism (5,7,8). A number of studies have indicated that COX-2 independent celecoxib-induced apoptosis may occur via inactivation of Akt signaling, increased production of ceramide or modulation of the mitochondria-mediated death pathway (2,9). Celecoxib is also known to cause changes in the expression of genes in the target cells. We reported previously that celecoxib treatment activated nuclear factor-kappaB (NF-κB) in cervical cancer cells, resulting in Fas death receptor expression and subsequent apoptosis, whereas inhibition of NF-κB partially blocked celecoxib-induced apoptosis in these cells (5). The identification of additional celecoxib-regulated genes will provide new insight into celecoxib-related signaling mechanisms, and may facilitate the development of new celecoxib-based anticancer strategies.

Here, we sought to identify genes that might be associated with celecoxib-induced apoptosis in cervical cancer cells. Analysis of a microarray cDNA-chip allowed us to identify several genes that were upregulated in celecoxib-treated cervical cancer cell lines (HeLa, CaSki and C33A). Among the upregulated genes, the growth arrest and DNA damage inducible gene (GADD153) was consistently elevated in all three cell lines.

GADD153, also known as CHOP [CEBP (CCAAT/enhancer-binding protein b) homology protein], has been demonstrated to be involved in growth arrest and apoptosis following DNA damage and a variety of stresses, such as nutrient deprivation and treatment with anticancer agents (10–13). We further evaluated the role of GADD153 expression and regulation in this system, providing important new insight into the anticancer effects of celecoxib.

Materials and methods

Cell lines and reagents

HeLa, CaSki and C33A cell lines were obtained from the American Type Culture Collection (Manassas, VA) and grown in RPMI1640 supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin (all from Invitrogen, Carlsbad, CA).

The NF-κB inhibitor (N-tosyl-L-phenylalanyl-chloromethyl ketone; TPCK) was purchased from Calbiochem (La Jolla, CA). Stock solutions were freshly prepared in dimethyl sulfoxide (DMSO; 0.001%) and added to the indicated final inhibitor concentrations. The final DMSO concentration was 0.001% and the same concentration was used as vehicle. DMSO alone (0.001%) was found to have no significant effect on cell function versus untreated cells. Propidium iodide was obtained from Sigma Chemical (St Louis, MO).

Anti-GADD153 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bak and Bax were obtained from Cell signaling (Beverly, MA). The anti-β-actin antibody (used as a loading control) was obtained from Sigma.

Abbreviations: COX-2, cyclooxygenase-2; CHOP, CEBP (CCAAT/enhancer-binding protein b) homology protein; GADD153, growth arrest and DNA damage inducible gene; NF-κB, nuclear factor-kappaB; siRNA, silencing RNA; TPCK, N-tosyl-L-phenylalanyl-chloromethyl ketone.
and the LipofectAMINE 2000 transfection reagents were purchased from Invitrogen.

cDNA microarray analysis
Total RNA was isolated with the TRIZOL reagent (Life Technologies, Gaithersburg, MD). Total RNA (40 μg) was labeled and hybridized to Human Apoptosis CHIP Version 1.1 (Takara Shuzo, Japan) as described previously (14). In brief, fluorescence-labeled target cDNA was prepared with a labeling kit (Macrogen, Korea) in the presence of fluorescent labeled dNTPs (Cy3 dUTP or Cy5 dUTP). Labeled target cDNA were hybridized to cDNA microarray for 16 h in 3x SSC at 65°C. Hybridized slides were washed at room temperature once in 0.5x SSC, 0.01% SDS for 5 min and again in 0.06x SSC for 5 min. The Cy3 and Cy5 signals were obtained using a confocal laser scanner, and fluorescence intensity was analyzed with the ImageGene v4.0 software (BioDiscovery Ltd, Swansea, UK). Data were normalized by non-linear regression (15).

Construction and transfection of the GADD153 expression vector
For transfection of the plasmid expression vector encoding human GADD153 the DNA sequencing containing the GADD153 open reading frame flanked by BamH1–Xho restriction sites was PCR amplified from HeLa cells primers designed to introduce the Kozak (underline) sequence (16) to increase translation (5'-GGG GAT CCA CCA TGG CAG CTG AGT CAT TGC CT and 5'-GCC TCG AGT CAT GCT TGG TGC AGA TTA CT). The resulting fragment was inserted into BamHI/XhoI-precut pcDNA3 (Invitrogen) to generate pcDNA3-GADD153. The desired sequence was confirmed by direct DNA sequencing.

For transfection, cervical cancer cells were grown to 70% confluence and transfected in serum-free medium for 6 h with LipofectAMINE 2000 (Invitrogen) and pcDNA3-GADD153 or empty vector (control). After 48 h, cells were harvested, washed twice with ice-cold phosphate-buffered saline (PBS), and processed for apoptosis analysis or western blot analysis.

Total RNA isolation and RT–PCR analysis
Total RNA was isolated as above and cDNAs were synthesized from 1 μg total RNA by using M-MLV reverse transcriptase (Invitrogen) with random hexamer priming. PCR was performed with specific primers (GADD153 sense 5'-GCT CTA GAG GCC TGC AGA GAT GGC-3', antisense 5'-GGA ATT CGG AGC TCA CGC CCC-3', β-actin sense 5'-ACA CTG CCA TCT ACG AGC-3', antisense 5'-AGG GGC CGG ACT CTG CAT ACT-3') using the following amplification conditions: 95°C for 3 min, followed by 95°C cycles for 1 min, and 58°C for 1 min, and 72°C for 1 min.

Real-time RT–PCR
Real-time quantitative PCR was performed in an iCycler IQ (Bio-Rad, Hercules, CA) using DNA Master SYBR Green I dye (Roche, Basel, Switzerland). Threshold cycles were measured at the end of each extension step, using the second-derivative method offered by the iCycler IQ optical system software (version 3.0a, Bio-Rad Laboratories). Melting curve analysis was performed to confirm and identify the peaks of interest in each sample. The results were normalized with regard to β-actin mRNA levels.

Western blotting
Protein extraction was performed as described previously (5): 50 μg of cell lysates was electrophoresed by 12% SDS–PAGE, transferred on to a polyvinylidene difluoride (PVDF) membrane and immunoblotted with the indicated antibodies. Bound antibodies were detected using appropriate peroxidase-coupled secondary antibodies and visualized by enhanced chemiluminescence (ECL) detection system (Amersham, Piscataway, NJ).

Construction of silencing RNA (siRNA)
The 21 nt siRNA duplexes (GADD153, GAUACUGCACAAGCGCAUGAU; GAPDH, GAACGCAGCGCCAGGUGAG) were purchased from Dharmacon Research (Lafayette, Co.). The siRNA oligonucleotides were transfected into cultured cells using Lipofectamine2000 according to the manufacturer’s recommendations. The cells were treated with celecoxib (50 μM), 36 h post-transfection, incubated for an additional 6 h and then harvested for apoptosis analysis or western blotting.

Flow cytometry analysis of apoptotic cells
Treated cells were trypsinized and washed with cold PBS, fixed with 70% ethanol and stored at −20°C until use. The fixed cells were stained with 20 μg/ml of propidium iodide containing 10 μg/ml RNaseA and then incubated at room temperature for 30 min in the dark. The DNA content of the cells (1 × 10^4/experimental group) was analyzed by FACSCalibur flow cytometer using the CellQuest analysis program (BD Biosciences NorthRyde, Australia). The sub-G1 population was considered to represent apoptotic cells.

Analysis of GADD153 promoter activities
The GADD153 luciferase reporter gene construct was kindly provided by Dr P. Fafournoux (17). We introduced 5’ mutations in the CE/EP-ATF and SP-1 sites using a site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. The mutant CE/EP-ATF and SP-1 CHOP promoter construct was generated by substitution of TAGA for GCCC (SP-1-mutant) or CAGATC for ATTGCA (CE/EP-ATF mutant). The results of mutant constructs were confirmed by sequencing. For experiments, cells were seeded in 12-well culture plates, 5 ×10^3 cells/well incubated overnight (4) and then transfected with a GADD153 luciferase reporter gene construct (0.4 μg/well) and pSV-β-galactosidase (0.1 μg/well) using LipofectAMINE 2000 reagent (Invitrogen). Cells were transferred to complete medium containing 10% FBS, 6 h post-transfection and 36 h post-transfection, cells were treated with celecoxib (50 μM), incubated for 6 h and then assayed for luciferase activities by using the Bioluminescent Reporter Gene Assay System (Tropix, Bedford, MA) according to the manufacturer’s instructions. The relative GADD153 promoter activities were normalized to that of β-galactosidase activity.

mRNA stability assay
The half-life of GADD153 mRNA was assayed as a measure of GADD153 mRNA stability. Cervical cancer cells were incubation with celecoxib for 6 h, and then treated with 1 μg/ml of the transcription inhibitor, actinomycin D (Act D; Sigma). Samples were harvested for RNA for the indicated time points and GADD153 mRNA levels were quantified by real-time quantitative PCR and normalized against β-actin. The half-life of GADD153 mRNA was calculated using the SigmaPlot software (SPSS, Chicago, IL).

Results
Celecoxib induces GADD153 expression in cervical cancer cells
We reported previously that celecoxib effectively induced apoptosis in cervical cancer cells (5). Here, we used a microarray cDNA screen for detecting genes involved in apoptosis to examine differential gene expression in celecoxib-treated cervical cancer cells (HeLa, CalCaSi and C33A) treated with 50 μM celecoxib for 6 h.

Out of a total of 225 different genes in the microarray were upregulated from 1.1 to ~10-fold in celecoxib-treated cell (Table 1). GADD153, a transcription factor involved in apoptosis, showed the most striking differential expression in all three cervical cancer cell lines and was thus chosen further for further study. To confirm the microarray results and examine celecoxib-induced regulation of GADD153 expression, we used western blotting analysis to examine celecoxib-induced changes in GADD153 protein levels over time in these cells. Consistent with the noted increase in the mRNA level, GADD153 protein levels increased in a time-dependent manner in celecoxib-treated cervical cancer cells (Figure 1).

Inhibition of celecoxib-induced apoptosis by silencing of GADD153 expression with siRNA
To determine the importance of the GADD153 in celecoxib-induced apoptosis, we used to siRNA methodology to silence the GADD153 gene. siRNA oligonucleotide specific for GADD153 or control oligonucleotide against GFP were transiently transfected into cervical cancer cells, which were then subjected to celecoxib treatment. Western blotting revealed that transfection of cervical cancer cells with 100 nM siGADD153 effectively suppressed celecoxib-induced increases in GADD153 expression (Figure 2A). Interestingly, transfection of siRNA to suppress GADD153 decreased the apoptotic sub-G1 fraction even in the presence of celecoxib (Figure 2B).
As shown in Figure 3, celecoxib increased the apoptotic sub-G₁ fraction of the cells up to \( \text{GADD153} \) to further determine the effect of \( \text{AIF4} \), apoptosis inhibitor 4; \( \text{cdc2} \), cell division cycle 2; \( \text{GADD153} \), DNA damage-inducible transcript; \( \text{cdc10} \), cell division cycle 10; \( \text{TP} \) p53, tumor protein p53; \( \text{TNFRAF} \), tumor necrosis factor (ligand) superfamily member 10; \( \text{cdc2-like1} \), cyclin-dependent kinase-like1; \( \text{p53} \), tumor protein p53; \( \text{TNFR}\alpha F \), tumor necrosis factor (ligand) DNA damage-inducible transcript; \( \text{cdc10} \), cell division cycle 10; \( \text{TP} \) kinase receptor type-2.

### Table I. List of apoptotic genes upregulated by celecoxib in three cervical cancer cell lines

<table>
<thead>
<tr>
<th>Gene name</th>
<th>HeLa (activation fold)</th>
<th>CaSki (activation fold)</th>
<th>C33A (activation fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIF4</td>
<td>1.72</td>
<td>2.11</td>
<td>1.18</td>
</tr>
<tr>
<td>Cdc2</td>
<td>2.73</td>
<td>1.26</td>
<td>1.19</td>
</tr>
<tr>
<td>GADD153</td>
<td>9.80</td>
<td>2.92</td>
<td>3.36</td>
</tr>
<tr>
<td>Cdc10</td>
<td>2.01</td>
<td>2.01</td>
<td>1.51</td>
</tr>
<tr>
<td>TP53</td>
<td>1.22</td>
<td>1.50</td>
<td>2.51</td>
</tr>
<tr>
<td>TNFRS</td>
<td>1.23</td>
<td>3.16</td>
<td>1.18</td>
</tr>
</tbody>
</table>

* \( \text{AIF4} \), apoptosis inhibitor 4; \( \text{cdc2} \), cell division cycle 2; \( \text{GADD153} \), DNA damage-inducible transcript; \( \text{cdc10} \), cell division cycle 10; \( \text{TP} \) p53, tumor protein p53; \( \text{TNFRAF} \), tumor necrosis factor (ligand) superfamily member 10; \( \text{cdc2-like1} \), cyclin-dependent kinase-like1; \( \text{IGF-2} \), insulin-like growth factor 2; \( \text{Rb-like2} \), retinoblastoma-like 2; \( \text{NF-x1} \), nuclear factor of kappa light polypeptide gene enhancer in B-0; \( \text{APAF} \), apoptotic protease activating factor; \( \text{NTK} \), neurotrophic tyrosine kinase receptor type-2.

*Mean value of replicated experiment in Takara double-spotted chip.

### Effect of ectopic \( \text{GADD153} \) overexpression on apoptosis in cervical cancer cell

To further determine the effect of \( \text{GADD153} \) on apoptosis, an expression plasmid containing the full-length cDNA of \( \text{GADD153} \) was transiently transfected into cervical cancer cells. As shown in Figure 3, \( \text{GADD153} \) overexpression increased the apoptotic sub-G₁ fraction of the cells up to 24–30%, whereas transfection with the empty vector was associated with the apoptotic sub-G₁ fraction <5%. These results indicate that the expression of \( \text{GADD153} \) is capable of inducing apoptosis in cervical cancer cells in the absence of other apoptotic signals.

### Celecoxib treatment increase the transcriptional activity and mRNA stability of \( \text{GADD153} \)

To examine whether celecoxib increases \( \text{GADD153} \) mRNA expression at the transcriptional or post-transcriptional level, we performed luciferase reporter gene assay and mRNA stability test.

### Celecoxib-induced activation of \( \text{GADD153} \) is independent of \( \text{NF-κB} \) signaling

Similar to \( \text{GADD153} \), activation of \( \text{NF-κB} \) is a hallmark or estrogen receptor (ER) stresses (19,20). In addition, \( \text{NF-κB} \) is transiently transfected cervical cancer cells with \( \text{GADD153} \) promoter constructs containing site-specific mutants of each cis-acting element. As shown in Figure 4B, the introduction C/EBP mutant construct significantly decreased the celecoxib-induced \( \text{GADD153} \) promoter activity. However, SP-1 mutant had no effect on \( \text{GADD153} \) promoter activity. These results suggest that C/EBP site is a necessary part of \( \text{GADD153} \) promoter for transcriptional activation caused by celecoxib.

Next, we tested whether mRNA stability was altered during celecoxib-induced \( \text{GADD153} \) upregulation. Cervical cancer cells were treated with celecoxib for 6 h (50 \( \mu \text{M} \)), and then replaced with fresh media containing celecoxib alone, Act D (1 \( \mu \text{g/ml} \)) + celecoxib, or Act D + vehicle. This time is considered 0 h. RNA was extracted at subsequent time points from each group, and relative mRNA levels were calculated by comparing \( \beta \)-actin-normalized values with the level observed in cells at time zero. Our results revealed that the half-lives of \( \text{GADD153} \) mRNAs in celecoxib-treated HeLa, CaSki and C33A cells were ~52, 90 and 70 min, respectively, while those in untreated cells were ~30, 39 and 43 min, respectively (Figure 4C). These results indicate that celecoxib appears to regulate \( \text{GADD153} \) mRNA expression at the transcriptional and post-transcriptional levels in cervical cancer cells.

![Figure 1. Induction of \( \text{GADD153} \) expression by celecoxib in cervical cancer cells. (A) Cervical cancer cells were treated with celecoxib (50 \( \mu \text{M} \)) for the indicated times. Qualitative real-time RT–PCR was used to assess cellular \( \text{GADD153} \) mRNA levels. The relative mRNA levels were normalized to that \( \beta \)-actin and ratio of \( \text{GADD153} \) to control mRNA was calculated (upper panel). Whole cell lysates (50 \( \mu \text{g} \)) were resolved by 12% polyacrylamide gel electrophoresis, transferred to nitrocellulose and probed with antibody against \( \text{GADD153} \) (lower panel). The blots were stripped and reprobed with antibody to \( \alpha \)-tubulin to verify as a loading control. The figures were representative of three independent experiments showing similar trend. The graph represents the mean ± SD of triplicate samples from three independent experiments.](image-url)
Fig. 2. Inhibition of celecoxib-induced apoptosis by silencing of GADD153 expression with small interfering RNA (siRNA). Cervical cancer cells were cultured in 6-well plate and transfected with control (sictrl, 100 nM) or GADD153 small interfering RNA (siGADD, 100 nM) for 36 h. After transfection, cells were treated with DMSO or 50 μM celecoxib for 6 h. (A) Whole cell lysates were prepared for western blotting analysis of GADD153 protein and α-tublin. (B) Each of these groups was analyzed by flow cytometry after propidium iodide staining. The percentage of sub-G1 phase cells was determined based on the DNA content histograms and represented as the mean ± SD of triplicate samples from three independent experiments.
known to promote apoptosis in various cancer cells (21–23), and we previously showed that celecoxib-induced apoptosis is mediated via NF-κB activation in cervical cancer cells (5). To determine whether celecoxib-induced GADD153 expression is mediated via NF-κB activation, we examined the effect of TPCK, a serine protease inhibitor that prevents degradation of Ik-Bα. We used same concentration of TPCK (0.1 μM) that previously showed to be sufficient to attenuate celecoxib-induced apoptosis in cervical cancer cells. However, our results revealed that pre-incubation of cells with 0.1 μM TPCK for 1 h prior to treatment with 50 μM celecoxib had no effect on GADD153 mRNA expression (Figure 5), suggesting that celecoxib-induced activation of GADD153 is independent of NF-κB signaling.

Celecoxib induces Bak expression in cervical cancer cells
Considerable evidence indicates that GADD153 mediates cellular response to oxidant injury, mainly by mediating ER stress (18,24,25). As some non-steroidal anti-inflammatory drugs (NSAIDs) can induce ER stress response including GADD153 (26), Bak has been found to be associated with the ER stress pathway (27,28). We therefore examined the relationship between GADD153 and Bak in celecoxib-induced apoptosis.

Western blotting analysis revealed that Bak was induced in celecoxib-treated cervical cancer cells (Figure 6A), with a time course that resembled the GADD153 response. To test whether GADD153 is responsible for the induction of Bak, we examined celecoxib-induced Bak expression, which was reduced by 50% in cells transfected siGADD (Figure 6B). This suggest that GADD153 may directly regulate at least a portion of celecoxib-induced Bak expression.

However, the observation that ~50% of celecoxib-induced Bak expression remained in siGADD153-treated cells suggest that celecoxib-induced Bak expression may be at least in partly mediated by other GADD153-independent mechanisms.

Discussion
We herein showed that GADD153 is critical for celecoxib-induced apoptosis of cervical cancer cells. Two lines of evidence support this conclusion: (i) silencing of the GADD153 gene by siRNA blocked celecoxib-induced apoptosis in cervical cancer cells and (ii) ectopic expression of GADD153 was sufficient to induce apoptosis in cervical cancer cells in the absence of additional apoptotic stimuli.

The GADD153, a member of the CCAAT/enhance-binding protein family of transcription factors, is a stress-induced, low molecular weight nuclear protein (13,29), also known as C/EBP homologous protein 10 (CHOP). GADD153 can negatively regulate C/EBP transcription factors that inhibit cell progression or can act as a positive regulator target genes (30). GADD153 expression has been associated with apoptosis in response to a number of stress stimuli, including anticancer agents, retinoic acid and nutrient deprivation (10–12). In addition to its function as a transcription factor, GADD153 has been shown to mediate apoptosis through non-transcriptional pathway (10). However, few studies have sought to establish a direct link between apoptosis and GADD153 expression.

Here, we showed that GADD153 has been shown to be upregulated during the apoptotic pathway. Interestingly, silencing of GADD153 expression blocked apoptosis in celecoxib-treated cervical cancer cells (Figure 2), indicating that GADD153 upregulation was not merely a consequence of apoptosis.

GADD153 expression may be regulated at both the transcriptional and post-transcriptional levels (10,31–33) and GADD153 mRNA stability is reportedly increased by nutrient deprivation and anticancer agents, such as paclitaxel and etoposide (34,35). Consistent with these previous findings, our luciferase and mRNA stability assays revealed that celecoxib treatment increased both the promoter activity of the GADD153 genes and the stability of the GADD153 mRNA. Additional luciferase assays using various deletional and mutant gene constructs revealed that the region between −649 and −249, including C/EBP-ATF binding site were required for celecoxib-induced GADD153 promoter activity. These results suggest that changes in GADD153 mRNA stability and promoter activity may be critically involved in celecoxib-induced apoptosis of cervical cancer cells, indicating that GADD153 may be a novel therapeutic target in cervical cancer cells.
Fig. 4. Transcriptional and post-transcriptional regulation of GADD153 mRNA in celecoxib-treated cervical cancer cells. Schematic representations of the GADD153 promoter deletion (A) and site-specific mutant (B) constructs are presented schematically. Cervical cancer cells were transfected with 400 ng of the promoter deletion of mutant constructs along with β-galactosidase vector. After 36 h, cells were incubated with celecoxib (50 μM) for 6 h and luciferase activities were measured. Results are represented in relative luciferase units (RLU). (C) GADD153 transcript stability in cervical cancer cells. The cells were treated with 50 μM celecoxib for 6 h and then transferred to medium containing celecoxib alone, Act D (1 μg/ml) + celecoxib, or Act D + vehicle. Total RNA was extracted at the indicated times, and GADD153 transcripts were measured and normalized with regards to the internal control, β-actin. Data are shown as mean ± SD.
We also examined the importance of NF-κB in GADD153-mediated apoptosis, because NF-κB was previously shown to downregulate GADD153, leading to decreased ER stress-induced apoptosis (36). In addition, we previously showed that celecoxib treatment increased the nuclear translocation of NF-κB, and enhanced apoptosis in cervical cancer cells, and that this effect could be blocked by addition of the NF-κB blocker, TPCK. Interestingly, the present study showed that GADD153 was upregulated irrespective of the DNA binding activity of NF-κB in celecoxib-treated cells (data not shown), and addition of NF-κB blockers to celecoxib-treated cervical cancer cells had no effect on GADD153 expression. These findings suggest there is no relationship between NF-κB and GADD153, and provide evidence that the regulatory
mechanism of GADD153 differ according to cell types and conditions.

Previous studies have reported association between Bcl-2-family members and GADD153-induced apoptosis (37,38). In other cells, GADD153 has important stimulatory interactions with members of the Bcl-2 family, while in other cells the induction of GADD153 results in Bcl-2 downregulation (37). Although celecoxib treatment does not alter Bcl-2 expression (data not shown), it is possible that other Bcl-2-related proteins are regulated in response to celecoxib. To examine this hypothesis, we investigated the expression levels of Bak which is associated with the endoplasmic reticulum (a key point of action for GADD153), and can induce apoptosis in combination with BH3-domain-only members of the Bcl-2-family such as Bid (39). Our results revealed that Bak expression was induced in celecoxib-treated cells, whereas transfection with siGADD153 reduced celecoxib-induced Bak expression by 50%. Our finding that Bak induction is less rapid than that of GADD153 and was blocked by siGADD153 seems to suggest that GADD153 may at least partly mediate celecoxib-induced Bak upregulation in cervical cancer cells.

In summary, we herein show that GADD153 is critical for celecoxib-induced apoptosis in cervical cancer cells. Celecoxib-induced GADD153 expression is mediated through a region between −649 and −249 of the GADD153 promoter, and requires an intact CEBP-ATF binding site.

As GADD153 is induced by chemotherapeutic drugs in other tumor types (34,35,40) and may play a key role in drug-induced apoptosis, these findings provide important new insight into signaling involved in GADD153-induced apoptosis by celecoxib and may facilitate the development of chemotherapeutic or chemopreventive strategies using celecoxib.

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Conflict of Interest Statement: None declared.

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