Modulation of Intracellular Signaling Pathways to Induce Apoptosis in Prostate Cancer Cells*

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An understanding of the molecular pathways defining the susceptibility of prostate cancer, especially refractory prostate cancer, to apoptosis is the key for developing a cure for this disease. We previously demonstrated that up-regulating Ras signaling, together with suppression of protein kinase C (PKC), induces apoptosis. Disregulation of various intracellular signaling pathways, including those governed by Ras, is the important element in the development of prostate cancer. In this study, we tested whether it is possible to modulate the activities of these pathways and induce an apoptotic crash among them in prostate cancer cells. Our data showed that DU145 cells express a high amount of JNK1 that is phosphorylated after endogenous PKC is suppressed, which initiates caspase 8 cleavage and cytochrome c release, leading to apoptosis. PC3 and LNCaP cells contain an active Akt. The inhibition of PKC further augments Akt activity, which in turn induces ROS production and accumulation of unfolded proteins in the endoplasmic reticulum, resulting in cell death. However, the concurrent activation of JNK1 and Akt, under the condition of PKC abrogation, dramatically augments the magnitude of apoptosis in prostate cancer cells.

It has been well documented that oncogenes (such as myc or ras) and their downstream effectors not only promote cell growth, but also, under certain circumstances, elicit programmed cell death or apoptosis (1–4). Mutational activation of the Ras gene is a key event in human cancer development (5–10). In the process of transformation, a persistent increase of Ras activity up-regulates its downstream effector pathways, leading to the phosphorylation and activation of pro-growth transcriptional factors (5–10). Despite its central involvement in cell growth and differentiation, we and others have demonstrated that treatment with PKC inhibitors can induce apoptosis in various types of cells overexpressing oncopgenic v-Ha or Ki-ras (11–16). In addition, stress-related kinases, such as c-Jun kinase (JNK)/p38 functions as Ras downstream effectors to initiate apoptosis (4).

Ras governs multiple downstream effector pathways, such as Raf/MAPK/ERK, PI3K/Akt, JNK/p38, and RalGDS (5–10). In response to mitogenic stimulation, Ras is activated, which in turn causes the plasma membrane translocation of the Raf/MAPK/ERK cascade, phosphorylation of transcription factors and oncogenes (5–10). The activation of PI3K or RalGDS has been shown to be involved in the regulation of cell growth. However, to promote growth-related activities, it has been shown that JNK1 acts as a pro-apoptotic signaling for the initiation of the apoptotic process, indicating that these pathways are involved in this process. The magnitude of apoptosis regulated by Ras remain not only be activated by Ras, but also by receptors or receptor protein-tyrosine kinases (24–27). Through activating Akt, PI3K has been shown to be involved in pro-survival activities (28–30). PTEN is an antagonist of PI3K, which is often mutated or deleted in various types of tumors, including prostate carcinoma (31–35). Increases of Akt activity have been observed to be associated with the development of prostate cancer, through affecting cell growth and angiogenesis (36). It has also been reported that the PI3K/Akt pathway regulates apoptosis, in which the kinases are either pro-apoptotic or anti-apoptotic, depending upon the types of stimuli and circumstances (37, 38). For example, Akt controls the status of several enzymes (such as NADPH oxidase) to promote the generation of ROS, the levels of which determine the outcomes (cell proliferation or death) (39, 40).

The ER serves as the site for synthesis, folding, modification, and trafficking of proteins, and plays a critical role in the maintenance of homeostasis (41–44). Pharmacological interference with ER function triggers the accumulation of misfolded proteins, resulting in the adaptive ER stress response program named the unfolded protein response (UPR) (45, 46). Activation of oncogenes has been demonstrated to be able to trigger the UPR (47–49). Studies also showed that, under persistent ER stress, the UPR plays a significant role in the initiation of apoptosis (50–52). The emerging evidence indicates that the ER, JNK, c-Jun NH2-terminal kinase; ER, endoplasmic reticulum; UPR, unfolded protein response; HO, heme oxygenase.

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‡ The abbreviations used are: PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; ER, endoplasmic reticulum; UPR, unfolded protein response; HO, heme oxygenase.
like other subcellular compartments, is a focal site for the initiation of programmed cell death pathways (50–52). Multiple ER stress-induced factors sensitize different intracellular targets to execute apoptotic programs. For example, GADD153 has been shown to heterodimerize with other CAAT/enhancer-binding protein family members and be able to induce apoptosis or regulate growth arrest (53–55). In mouse embryonic fibroblasts, the up-regulation of GADD153 correlates with the onset of PUMA- or NOXA-induced cell death (51).

We previously demonstrated that Ras mutation, together with abrogation of PKC, are synthetically lethal (11–16). Therefore, in this study, we tested whether abnormalities in Ras downstream effectors are able to induce apoptosis under PKC-deficient conditions in prostate cancer cells. The results demonstrated that the cooperation of JNK1, Akt, and PKC sensitizes prostate cancer cells to apoptosis through the activations of caspase 8 and the up-regulation of ROS.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—LNCaP, PC3, and DU145 cells are human prostate cancer cells (obtained from Dr. Z. Lou, Boston University School of Medicine) and cultured in RPMI1640 medium containing 10% fetal calf serum (Invitrogen). The v-Ha-ras, JNK, or active Akt are inserted in a retroviral vector.

For the suppression of GADD153, 20 μM sense (as control) or antisense oligos were added into cell cultures for 48 h, and subsequently the cells were re-fed with medium and a new dose of the oligos every 2 days. Antibodies used in the study were from Santa Cruz Biotechnology. The anti-Ras and caspase 8 antibodies were from Cell Signaling Technology. The anti-Akt antibody was from Cell Signaling Technology. The anti-PKC antibody was from Calbiochem. JNK inhibitor I is a cell-permeable, biologically active peptide, which inhibits JNK (Calbiochem).

**DNA Fragmentation Analysis**—Flow cytometric analysis was performed using a FACScan machine (BD Biosciences). The data analysis was performed using Cell-Fit software program (BD Biosciences). Cell-Fit receives data from the flow cytometer and provides real-time statistical analysis, computed at 1-s intervals, and also discriminates doublets or adjacent particles. Cells with sub-G0-G1 DNA contents after staining with propidium iodide were counted as apoptotic cells. In brief, following treatments, cells were harvested and fixed in 70% ethanol. Afterward, cells were washed with 0.1% Triton X-114, 0.5% sodium deoxycholate, 0.1% SDS, containing 1 mM leupeptin, 1 mg/ml pepstatin A) on ice for 30 min. The total protein concentrations in the cell lysates were normalized and adjusted to 0.4 M NaCl, 0.5% deoxycholate, and 0.05% SDS for immunoblotting (56). The samples were separated on a 10% SDS-PAGE gel and subsequently transferred to a nitrocellulose membrane.

**Measurement of Ral Activation**—After treatments, cells were lysed in a buffer containing 25 mM HEPES, pH 7.5, 150 mM NaCl, 1% lecithin CA-630, 0.25% sodium deoxycholate, 10% glycerol, and 25 mM NaF (56). Protein was normalized to 1 mg/ml and precipitated by mixing 1 mg of cell lysate with 20 μg of Ral-RBD overnight at 4 °C. The complexes were washed 5 times in lysis buffer, and then separated on a 12.5% SDS-PAGE gel. Proteins were transferred onto a polyvinylidene difluoride membrane and immunoblotted with a pan-Ras antibody ( Oncogene Research Products).

**Measurement of ROS**—Cells, after the treatments, were washed with ice-cold phosphate-buffered saline and resuspended in 5 μg/ml of 2’,7’- dichlorodihydrofluorescein diacetate (Molecular Probes). Samples were incubated for 10 min at room temperature and analyzed immediately by a flow cytometer (57).

**Preparation of Subcellular Fractions**—After treatments, cells were harvested, resuspended in 1× phosphate-buffered saline containing 1% Triton X-114 lysis buffer, and incubated for 30 min. The lysates were collected as the cytosolic fraction. The supernatant was collected as the perinuclear fraction.

**RESULTS**

**Induction of Apoptosis in Prostate Cancer Cells after Downregulation of PKC**—We and others have demonstrated that oncogenic Ras is able to induce human or mouse fibroblasts or lymphocytes to undergo apoptosis once endogenous PKC is suppressed (11–15). In this process, multiple Ras downstream effectors are involved for transmitting apoptotic signaling. Here, we tested whether it is possible to induce apoptosis in various prostate cancer cells with or without expressing v-Ha-ras following treatment with GO6976. A v-Ha-ras was transiently introduced into prostate epithelial PrEC cells or prostate cancer LNCaP, PC3, DU145, and HPV7 cells. After transfection, a high level of Ras expression was detected in all transfectants by immunoblotting (Fig. 1a). Subsequently, the cells with or without expressing v-ras were treated with GO6976 (0.1 μm) for 24 h and a DNA fragmentation assay was conducted to determine the occurrence of apoptosis (Fig. 1b). More than 40% of PrEC/ras and prostate cancer cell lines expressing v-ras underwent apoptosis in response to PKC down-regulation. Interestingly, 25% of PC3 or DU145 cells and 15–18% of LNCaP cells had fragmented DNA following treatment. In comparison, apoptosis did not occur in PrEC or HPV7 cells after the addition of GO6976. The results indicate that persistent activation of Ras, together with suppression of PKC, can induce apoptosis in normal prostate epithelial or cancer cells.
Because some prostate cancer cell lines were moderately susceptible to apoptosis in response to GO6976 treatment, it indicates that the Ras-related, apoptotic pathway(s) is activated in these cells. Therefore, we examined the expression or activation status of Ras downstream effectors in PrEC and prostate cancer cell lines by immunoblotting (Fig. 2). ERK1/2 was detected in all cell lines and the phosphorylated forms of the kinases were only present in DU145 cells, but not in PrEC or other prostate cancer cell lines (Fig. 2a). Anti-JNK1 antibody revealed a high level of this stress-related kinase in DU145 cells only (Fig. 2b). It is known that PTEN mutation or deletion is often detected in prostate cancer cells, which leads to the up-regulation of PI3K/Akt signaling (31–33). We then tested the activation status of Akt in these cells using the anti-phosphorylated Akt antibody (Fig. 2c). Although a similar amount of Akt was present in all cell lines, however, the antibody detected a high level of the phosphorylated Akt in PC3 cells, and a moderate amount of the active kinase in LNCaP cells, which may reflect that PC3 cells are in more advanced stages of the malignancy than LNCaP cells. The active form of Akt was undetectable in PrEC, DU145, or HPV7 cells. The expression of p38 or activation of Ral was also tested using either anti-p38 antibody or Ral/RBD-glutathione S-transferase fusion protein. Only a basal-line expression of p38 was present in all cell lines tested, and there was no association of Ral with the fusion protein (Fig. 2, d and e).

**Down-regulation of PKC Elicits JNK Activation and Caspase Cascade in DU145 Cells**—Activation of JNK has been shown to be involved in apoptosis induced by various apoptotic stimuli (16). We previously reported that JNK1 functions as an intermediary during Ras-mediated apoptosis to initiate caspase 8-regulated caspase cascade (16). Because the addition of GO6976 elicited a moderate magnitude of apoptosis in DU145 cells that expressed an elevated level of JNK1, we tested the activation status of JNK1 in the cells in the presence or absence of GO6976 treatment using the anti-phosphorylated JNK anti-
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**FIGURE 3.** JNK1 and caspase 8/cytochrome c are activated after PKC suppression.

**a** JNK1 expression was examined (Fig. 4a). JNK1 was not activated in untreated cells, but was completely blocked by the addition of JNK inhibitor (Fig. 4c). Down-regulation of PKC Causes the Accumulation of ROS and UPR in LNCaP and PC3 Cells—Increase of Ras activity in cells has been linked to the augmentation of ROS production, which is required for the transformation process (58). Studies have also shown that oncogenic Ras, under the condition of PKC abrogation, dramatically up-regulates ROS production that in turn participates in the apoptotic process (59, 60). Because JNK1 expression and activity were not increased in LNCaP and PC3 cells and these cells were still susceptible to PKC suppression-induced apoptosis, it led us to test the redox state in these cells (Fig. 5a). A moderately elevated ROS was present in untreated LNCaP and PC3 cells. In contrast, DU145 and HPV7 cells had a very low amount of ROS. The introduction of v-ras into HPV7 cells moderately increased ROS production, which is consistent with others’ findings that Ras is able to up-regulate ROS production (59, 60). After treatment with GO6976, the amount of ROS in LNCaP or PC3 cells as well as in HPV7 cells expressing v-ras, was further increased. We

MAPK/ERK1/2 pathway or cooperation between ERK and JNK. To clarify the role of ERK1/2 in the regulation of apoptosis and to define the requirement of JNK1 in this process, PD98059 (an inhibitor for MAPK/ERK1/2 pathway) or JNK inhibitor was used prior to PKC suppression in DU145 cells (Fig. 4).

The cleaved, active caspase 8 was present in GO6976-treated DU145 cells after suppression of the MAPK/ERK1/2 pathway, but was absent following the addition of JNK inhibitor (Fig. 4a). Furthermore, cytochrome c was released into the cytosol of DU145 cells after suppression of GO6976 in the absence of MAPK/ERK1/2 signaling, which happened in the same cells interrogating JNK1 activity (Fig. 4). The results of the DNA fragmentation assay were consistent, in which apoptosis occurred in treated DU145 cells in the presence of PD98059, but was completely blocked by the addition of JNK inhibitor (Fig. 4c).

Down-regulation of PKC Causes the Accumulation of ROS and UPR in LNCaP and PC3 Cells—Increase of Ras activity in cells has been linked to the augmentation of ROS production, which is required for the transformation process (58). Studies have also shown that oncogenic Ras, under the condition of PKC abrogation, dramatically up-regulates ROS production that in turn participates in the apoptotic process (59, 60). Because JNK1 expression and activity were not increased in LNCaP and PC3 cells and these cells were still susceptible to PKC suppression-induced apoptosis, it led us to test the redox state in these cells (Fig. 5a). A moderately elevated ROS was present in untreated LNCaP and PC3 cells. In contrast, DU145 and HPV7 cells had a very low amount of ROS. The introduction of v-ras into HPV7 cells moderately increased ROS production, which is consistent with others’ findings that Ras is able to up-regulate ROS production (59, 60). After treatment with GO6976, the amount of ROS in LNCaP or PC3 cells as well as in HPV7 cells expressing v-ras, was further increased. We
then tested whether the expression of heme oxygenase (HO-1, a ROS modulator) is altered after treatment using immunoblotting (Fig. 5b). HO-1 could not be detected in LNCaP, PC3, or HPV7/ras cells under normal growth conditions and induced after treated with GO6976. The data indicate that a moderate increase of ROS under normal growth conditions is unable to induce HO-1 in these cells, but the ROS modulator is up-regulated by GO6976 treatment. These findings also pointed to the possible role of Akt signaling in the perturbation of the equilibrium of the intracellular redox state.

Activation of PI3K/Akt or MAPK, under certain circumstances or in different types of cells, can cause ER stress and subsequent unfolded protein accumulation, which triggers the UPR (49). Under such conditions, cells induce ER chaperone proteins to relieve protein aggregation and activate the proteasome machinery to degrade misfolded proteins. However, the UPR induced by persistent ER stress often switches on the apoptotic machinery (49). GADD153 is often up-regulated during ER stress, the induction of which is closely correlated with the onset of apoptosis (53–55). To determine whether PKC suppression could induce GADD153 expression in prostate cancer cells, Western blot analysis was performed (Fig. 5c, upper panels). GADD153 was detected by the antibody in LNCaP or PC3 cells, but not in DU145 cells following GO6976 treatment. Consistently, the level of this ER protein was increased in LNCaP or PC3 cells, but not in DU145 cells (Fig. 5c, lower panels).

Akt Is Required for the Induction of Apoptosis in GO6976-treated LNCaP or PC3 Cells—LNCaP or PC3 cells, in the absence of JNK1 activation, were still sensitive to GO6976 treatment for the induction of apoptosis. Also, PC3 cells with a high amount of phosphorylated Akt are more susceptible to apoptosis than LNCaP cells that express a moderately elevated level of active Akt (see Figs. 1 and 2). These led us to further investigate the role of Akt activation in the initiation of apoptosis. We first examined whether GO6976 treatment further affects the level of Akt phosphorylation in PC3 or LNCaP cells by immunoblotting. The phosphorylated Akt was present in untreated cells, in which the level of the activated kinase in PC3 cells was higher than that in LNCaP cells (Fig. 6a). The addition of the inhibitor further proportionally increased the amounts of phosphorylated Akt in both cell lines. Next, we tested the susceptibility of GO6976-treated LNCaP, PC3, and DU145 cells to apoptosis following suppressing the Akt signaling pathway by the Akt inhibitor, ROS production by NCA (a ROS inhibitor), or GADD153 by GADD153 antisense oligos (Fig. 6b). These chemical or genetic inhibitors blocked the apoptotic process in LNCaP and PC3 cells, and played no role in DU145 cells. To further test the effect of Akt on the onset of apoptosis induced by PKC suppression and define the possible role of MAPK signaling in this process, a constitutively active Akt or V12S35ras that preferentially binds to and activates Raf/MAP kinase was transiently introduced into prostate cancer cells. Following PKC down-regulation, the constitutive activation of the Akt pathway had no further influence on the induction of apoptosis in LNCaP or PC3 cells. In contrast, the up-regulation of Akt signaling dramatically augmented the magnitude of apoptosis in DU145 cells. Again, activation of the MAPK pathway by V12S35ras did not affect apoptosis triggered by GO6976 treatment. These data suggest that Akt, but not MAPK, takes part in apoptosis.
the regulation of apoptosis triggered by PKC suppression in LNCaP and PC3 cells.

DISCUSSION

It is known that cells expressing oncogenic Ha- or Ki-ras are susceptible to apoptosis following the suppression of endogenous PKC (11–15). In this Ras-mediated apoptotic process, JNK1 activation is required (16). It has also been reported that multiple Ras downstream effector pathways are involved in the regulation of this cell death program (13). In this study, using various prostate cancer cell lines with different expression levels or activation status of several important signal transducers, we assessed the contribution of each Ras-related pathway to the induction of apoptosis. The study discloses a regulatory network for the induction of apoptosis in prostate cancer, in which the concurrent activation of Akt and JNK1 pathways synergize with PKC suppression to induce a full execution of programmed cell death. In this process, Akt signals to a machinery controlling the intracellular redox state, which in turn elicits ER stress and the UPR. JNK1 functions in concert with PKC down-regulation on a separate pathway to induce apoptosis through recruiting caspase activity.

JNK, as one of Ras downstream effectors, phosphorylates the transcriptional factor c-Jun, which further participates in regulation of cell growth, differentiation, or apoptosis. The increase of JNK1 activity by tumor necrosis factor or Fas receptor has been shown to be responsible for the induction of apoptosis (61, 62). In response to the ligation between the ligand and receptor, a death-induced signaling complex is formed and subsequently activates JNK1, leading to caspase 8-mediated caspase cascade. A similar pattern of caspase cascade triggered by JNK1 occurs through a mechanism that perturbs the intracellular redox equilibration that in turn triggers the UPR, resulting in apoptosis. Thus, the data presented here suggest that Akt, JNK1, and PKC not only participate in the regulation of cell growth-related activities, but also function as important apoptotic mediators in the initiation of apoptosis in prostate cancer. Moreover, the concurrent activation of JNK1 and Akt, under the condition of PKC abrogation, achieves a full execution of programmed cell death.

Persistent increases of Ras activity trigger a wide spectrum of cellular responses, leading to transformation. These responses are often related to the oncogenic activities of Ras in the promotion of human malignancy. Recently, we and others have discovered that, under certain circumstances, oncogenic Ras is able to induce apoptosis, which has drawn attention for its therapeutic potential. Ras has been known to function through binding to multiple effector proteins that subsequently activate distinct downstream signaling pathways (5–10). It has also been shown that, upon PKC suppression, JNK1 activity is required for the formation of the death-inducing complex (16). However, the suppression of JNK only accounted for 40% reduction of Ras-mediated apoptosis (16). In this study, using various prostate cancer cell lines with different expression levels or activation status of several important signal transducers, we assessed the contribution of each Ras-related pathway to the induction of apoptosis. The study discloses a regulatory network for the induction of apoptosis in prostate cancer, in which the concurrent activation of Akt and JNK1 pathways synergize with PKC suppression to induce a full execution of programmed cell death. In this process, Akt signals to a machinery controlling the intracellular redox state, which in turn elicits ER stress and the UPR. JNK1 functions in concert with PKC down-regulation on a separate pathway to induce apoptosis through recruiting caspase activity.

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during Ras-mediated apoptosis (16). Studies also demonstrated that JNK1 is able to inactivate the ASK1 inhibitor, thioredoxin, which in turn mobilizes ASK1 signaling to up-regulate ROS for the induction of apoptosis (63). Using DU145 containing a high amount of JNK1 as well as LNCaP and PC3 cells that express a very low or undetectable level of the protein, we demonstrated that JNK1 activation plays no role in ROS accumulation during this PKC suppression-triggered apoptosis. Instead, like in Fas-induced apoptosis, this kinase is crucial for the initiation of caspase 8 cleavage and cytochrome c releasing.

ROS is an important intracellular signal transducer of growth factors (58–60). In response to abnormally persistent ligations of growth factor receptors, a moderate increase of intracellular ROS was induced, which has been shown to be required for cellular transformation by altering the structure of the cytoskeleton and further inducing a transformed phenotype in a Rac-dependent fashion (58). In PC12 cells, Ras has also been suggested to up-regulate ROS production upon epidermal growth factor stimulation (64). Furthermore, studies demonstrated that oncogenes can perturb the equilibrium of the intracellular redox state and cause DNA single strand breaks, which in turn disrupts genetic integrity (57). Despite regulating cell proliferation and transformation, the increase of ROS production has also been suggested to play an obligatory role in the induction of apoptosis by various apoptotic stimuli (59, 60). In NFκB-regulated programmed cell death, ROS accumulation precedes...
mitochondrial depolarization and caspase activation (65). Our study demonstrated that the activation of Akt in LNCaP or PC3 cells moderately induces ROS production, which may be utilized for tumor maintenance or growth (57). Notably, the inhibition of PKC further augments the level of Akt phosphorylation in these tumor cells, which coincides with a further up-regulation of ROS production. It is possible that PKC and Akt function in an opposite way to maintain the intracellular redox state. After lifting the negative control rendered by PKC, activated Akt disrupts the balance of the intracellular redox state, leading to high amounts of ROS accumulation.

ER stress has been shown to be activated by alternations in proteins and lipid metabolism that can cause the accumulation of unfolded proteins (50–52). Dysregulation of ER stress programs, such as the UPR, can elicit cytoprotective or cytotoxic reactions, depending upon the severity of the stress or other cellular regulators. GADD153 is a transcriptional factor and often induced upon ER stress (36–38). Under the GADD153-deficient condition, cells presented a resistance to ER stress-induced apoptosis, indicating the importance of GADD153 in this process (50–52). Our study showed that ER stress chaperones, including GADD153, are induced in response to PKC suppression in LNCaP and PC3 prostate cancer cells. Using the antisense oligos, we identified that GADD153 functions downstream of Akt and acts as a crucial apoptotic modulator. In this process, a significant accumulation of unfolded proteins occurred, which subsequently elicited apoptosis. PKC inhibition, surpass the buffering turn trigger the cell death program, indicating that the ER functions as a sensor to detect changes in the cellular microenvironment.

Taken together, the up-regulation of the JNK1 or Akt pathways often induces apoptosis in these tumor cells, which coincided with a further up-regulation of ROS production. It is possible that PKC and Akt function in an opposite way to maintain the intracellular redox state, leading to high amounts of ROS accumulation.

ESR stress has been shown to be activated by alternations in proteins and lipid metabolism that can cause the accumulation of unfolded proteins (50–52). Dysregulation of ES stress programs, such as the UPR, can elicit cytoprotective or cytotoxic reactions, depending upon the severity of the stress or other cellular regulators. GADD153 is a transcriptional factor and often induced upon ES stress (36–38). Under the GADD153-deficient condition, cells presented a resistance to ES stress-induced apoptosis, indicating the importance of GADD153 in this process (50–52). Our study showed that ES stress chaperones, including GADD153, are induced in response to PKC suppression in LNCaP and PC3 prostate cancer cells. Using the antisense oligos, we identified that GADD153 functions downstream of Akt and acts as a crucial apoptotic modulator. In this process, a significant accumulation of unfolded proteins occurred, which subsequently elicited apoptosis. PKC inhibition, surpass the buffering turn trigger the cell death program, indicating that the ER functions as a sensor to detect changes in the cellular microenvironment.

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