Leukotriene B4 receptor antagonist LY293111 induces S-phase cell cycle arrest and apoptosis in human pancreatic cancer cells

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We have previously shown that the leukotriene B4 receptor antagonist, LY293111 inhibits proliferation and induces apoptosis in human pancreatic cancer cells both *in vitro* and *in vivo*. In the current study, we investigated the molecular mechanisms of LY293111-induced apoptosis and cell cycle arrest. Two human pancreatic cancer cell lines were used in this study, MiaPaCa-2 and AsPC-1. Cell cycle analysis by flow cytometry showed a dramatic increase in the percentage of apoptotic cells as well as S-phase arrest after treatment with 250 nmol/l LY293111 for up to 48 h. Western blotting indicated that LY293111 treatment induced cytochrome *c* release from the mitochondria into the cytosol, accompanied by caspase-9, caspase-7 and caspase-3 activation, and cleavage of poly ADP-ribose polymerase. Caspase-8 was not activated by LY293111. A decrease was found in the expression of the antiapoptotic proteins, Bcl-2 and Mcl-1, and an increase in the proapoptotic protein, Bax. LY293111 reduced the expression of CDK2, cyclin A and cyclin E, consistent with the S-phase arrest observed in these cells. The expression of cyclin-dependent kinase inhibitors, p21 and p27 was not affected by LY293111 treatment. In conclusion, LY293111 induces apoptosis in human pancreatic cancer cells through the mitochondria-mediated pathway. LY293111 also induces S-phase arrest with downregulation of CDK2, cyclin A and cyclin E. Blockade of leukotriene B4 metabolic pathway may provide a novel treatment for human pancreatic cancer. *Anti-Cancer Drugs* 18:535–541

Anti-Cancer Drugs 2007, **18**:535–541

Keywords: apoptosis, leukotriene B4 receptor antagonist, LY293111, pancreatic cancer, S-phase arrest

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Sponsorship: These studies were supported by grants from the NCI SPORE program (P50 CA72712), the Lustgarten Foundation for Pancreatic Cancer Research, the Rolfe Foundation for Pancreatic Cancer Research and the Lilly Research Laboratories.

Received 20 February 2006 **Accepted** 17 June 2006

Introduction

Apoptosis is a cellular suicide program critical for the normal organ development and function [1,2]. Abnormalities in the control of this pathway contribute to cancer [3]. The apoptosis pathway consists of three major components: the Bcl-2 family of proteins, a family of cysteine proteases called caspases and the apoptosis activating factor (Apaf-1) that relays the signals integrated by Bcl-2 family proteins to the caspases [3–6]. Biochemical activation of these key components of the cell death program is responsible for the morphological changes observed in apoptosis, including mitochondrial damage, nuclear membrane breakdown, DNA fragmentation, chromatin condensation and the formation of apoptotic bodies [7,8]. These changes are distinct from necrosis both morphologically and biochemically.

There are two major apoptotic pathways. (1) Death receptor-mediated apoptosis, which is initiated by triggering of cell surface death receptors of the tumor necrosis factor receptor superfamily [9,10]. Caspase-8 is activated following its recruitment to a trimerized receptor–ligand complex via adaptor molecules such as the Fas-associated death domain. (2) Mitochondrial-mediated apoptosis, which is caused by chemicals, irradiation and growth factor deprivation, and results in disruption of mitochondria and release of cytochrome *c* into the cytosol. Cytosolic cytochrome *c* binds to Apaf-1 to form the apoptosome complex, which recruits and activates caspase-9 [9,10]. The release of cytochrome *c* is also regulated by Bcl-2 family proteins. Proapoptotic Bcl-2 family members, such as Bax and bad, promote cytochrome *c* release, whereas the antiapoptotic members, such as Bcl-2, Bcl-x_L, and Mcl-1, inhibit its release. Activated caspase-8 and caspase-9 can then converge to a common pathway, which involves the activation of effector caspases, i.e. caspase-3, caspase-6 and caspase-7, which are responsible for the cleavage of cellular substrates, such as lamin, actin, Rb and poly ADP-ribose polymerase (PARP), and subsequently inducing characteristic apoptotic changes [11,12].

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Lipoxygenases (LOXs) are enzymes involved in the metabolism of the arachidonic acid, a fatty acid derived from the membrane phospholipids by the enzyme phospholipase A2. Three different isoforms of LOX exist in the cells, i.e. 5-LOX, 12-LOX and 15-LOX, which add a molecule of oxygen to the arachidonic acid to produce 5-HETE, 12-HETE and 15-HETE, respectively [13–15]. The importance of the 5-LOX pathway is that it is the only pathway in the cells to produce leukotrienes (LTs), such as LTB4, LTC4, LTD4 and LTE4, which constitute a class of potent mediators of inflammation and anaphylaxis. Among these, LTB4 has been widely implicated in the pathogenesis of several inflammatory diseases, such as asthma, psoriasis, rheumatoid arthritis and inflammatory bowel disease [16,17]. Previous studies in our laboratory and that of others have shown the importance of LOX pathways in the tumorigenesis of multiple human cancers, including pancreatic cancer [18,19]. Various LOX inhibitors block proliferation and induce apoptosis in human prostate, breast, colon, gastric and pancreatic cancer as well [18–22]. The molecular mechanisms involved in LOX inhibitor-induced apoptosis have been extensively studied in recent years. The induction of apoptosis in gastric cancer cells by the 12-LOX inhibitor, baicalein involves a decrease in Bcl-2 expression, and activation of caspase-7 and caspase-3 [23]. The 5-LOX-activating protein inhibitor MK886 also causes apoptosis in human gastric cancer cells, with increased levels of Bax and the cyclin-dependent kinase (CDK) inhibitor, p27 and the activation of caspase-3 [24]. In breast cancer, inhibition of 5-LOX by NDGA or MK886 induces apoptosis both *in vivo* and *in vitro*, with decreased Bcl-2 and increased Bax expression [25]. The 12-LOX inhibitor baicalein induces apoptosis in PC3 and DU-145 prostate cancer cell lines, and the mechanisms involve decreased phosphorylation of protein kinase B (Akt), loss of survivin, and activation of caspase-3 and caspase-7. Baicalein also decreases the expression of Bcl-2 and Bcl-x_L and causes a shift of Bcl-2/Bax level, favoring apoptosis in these cells [26]. The LOX inhibitors, NDGA and *N*-benzyl-*N*-hydroxy-5-phenylpentanamide, as well as 12-LOX antisense oligonucleotides cause apoptosis in rat Walker 256 (W256) carcinoma cells [27]. This apoptosis is related to downregulation of Bcl-2 and a dramatic decrease of the Bcl-2/Bax ratio. This was suppressed by overexpression of Bcl-2 [27].

Our previous studies have also shown the importance of LTB4 signaling in the growth of human pancreatic cancer. Blockade of LTB4 signal pathway using the selective LTB4 receptor antagonist, LY293111, inhibited proliferation and induced apoptosis in human pancreatic cancer cell both *in vitro* and *in vivo* [28]. LY293111 inhibits development of metastases as well as the growth of primary tumor in a novel orthotopic model of pancreatic cancer in the mouse and also significantly enhances the effect of gemcitabine in this model [29]. In the present study, we investigated the molecular mechanism of LY293111-induced apoptosis and cell cycle arrest in human pancreatic cancer cells. This will help us to understand the mechanism of LY293111 as a novel therapeutic agent for this malignant disease.

Methods

Materials

Dulbecco's modified Eagle's medium and minimal essential medium, penicillin–streptomycin solution, and trypsin–ethylene diaminetetraacetic acid (EDTA) solution were purchased from Sigma (St Louis, Missouri, USA). Fetal bovine serum (FBS) was from Atlanta Biologicals (Norcross, Georgia, USA). The LTB4 receptor antagonist, LY293111, was a kind gift from Drs Jake Starling and Jerome Fleisch from Lilly Research Laboratories (Indianapolis, Indiana, USA). Polyclonal caspase-3, monoclonal caspase-7, caspase-8 and caspase-9 antibodies were from Pharmingen (San Diego, California, USA). The monoclonal PARP antibody was from Biomol (Plymouth Meeting, Pennsylvania, USA). The monoclonal Bcl-2, Mcl-1, Bax, CDK2, cyclin A, cyclin E, p21 and p27 antibodies were from Santa Cruz Biotechnology (Santa Cruz, California, USA). Protein assay reagents were from Bio-Rad (Hercules, California, USA).

Human pancreatic cancer cell lines and cell culture

MiaPaCa-2 and AsPC-1 human pancreatic cancer cells used in this study were purchased from the American Type Culture Collection (Rockville, Massachusetts, USA). MiaPaCa-2 cells were grown in Dulbecco's modified Eagle's medium and ASPC-1 cells were grown in minimal essential medium supplemented with 10% FBS in a humidified atmosphere of 95% O_2 and 5% CO_2 at 37°C. The cells were regularly seeded into T75 flask with media changed every other day.

Cell cycle analysis by flow cytometry

MiaPaCa-2 and AsPC-1 pancreatic cancer cells were cultured in T75 flasks with media containing 10% FBS. After reaching 50–60% confluence, the cells were serum-starved for 24 h, and then treated with 250 nmol/l LY293111 for 24 and 48 h. At the end of the treatment, the cells were harvested with trypsin–EDTA solution to produce single-cell suspensions. The cells were then centrifuged at 1000 *g* for 10 min. The pellets were washed twice with ice-cold phosphate-buffered saline (PBS), and resuspended in 0.5 ml PBS. Five milliliters of ice-cold 70% ethanol were added to each cell suspension and incubated for 12 h at 4°C. The cells fixed in ethanol were centrifuged at 1000 *g* for 10 min and the subsequent pellets were washed twice with ice-cold PBS. The final cell pellets were reconstituted in 1 ml Telford reagent and horizontally shaken for 1 h in the dark at room temperature. The red fluorescence of the single events

was recorded using a laser beam with 488 nm for excitation and 610 nm as emission, to measure the DNA index.

Western blotting

Following treatment of MiaPaCa-2 and AsPC-1 pancreatic cancer cells with or without LY293111, cells were scraped into 1 ml lysing buffer [20 mmol/l Tris–HCl, pH 7.4, 2 mmol/l sodium vanadate, 1.0 mmol/l sodium fluoride, 100 mmol/l NaCl, 2.0 mmol/l phosphate substrate, 1% NP-40, 0.5% sodium deoxycholate, 25 μg/ml each aprotinin and leupeptin, 25.0 μg/ml pepstatin, 2.0 mmol/l each EDTA and ethylene glycol-bis(*b*-aminoethyl ether)] at 4°C. Cell lysates were clarified by microcentrifugation at 12 000 *g* following incubation at 4°C for 25 min. The supernatants were recovered and their protein concentrations measured using the Bio-Rad protein assay reagent. Equivalent amounts of cell lysate protein (30 μg) were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (15%). Proteins were transferred to nitrocellulose membranes by electroblotting using a Bio-Rad mini semi-dry transfer blotting apparatus. Membranes were subsequently blocked in Tris-buffered saline containing 0.1% Tween-20, then incubated with appropriate polyclonal or monoclonal antibodies in 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20 overnight at 4°C. The membrane-bound protein–antibody complex was reacted with horseradish peroxidase-conjugated goat anti-rabbit antibody at 1:2000 dilution (for polyclonal antibodies) and anti-mouse antibody at 1:2000 dilution (for monoclonal antibodies) for 1 h at room temperature. The membranes were then detected by chemiluminescence involving treatment of membrane with LumiGlo reagent and light emission was captured on Kodak X-ray films.

Statistical analysis

Data were analyzed by analysis of variance with Dunnett's corrections for multiple comparisons. This analysis was performed with the Prism software package (GraphPad, San Diego, California, USA).

Results

LY293111 induces apoptosis and S-phase arrest in pancreatic cancer cells *in vitro*

The selective LTB4 receptor, LY293111, has been shown previously to inhibit proliferation and induce apoptosis of multiple human pancreatic cancer cells. After 250 nmol/l LY293111 treatment for 24 and 48 h, cell cycle analysis using flow cytometry revealed a marked increase in the percentage of apoptotic cells, represented by the sub-G_0/G_1 fraction. This fraction increased from 1.8 to 18.5 and 42.8% in MiaPaCa-2 cells and from 0.8 to 13.5 and 31.4% in AsPC-1 cells, respectively, at 24 and 48 h (Table 1). We also observed a significant S-phase arrest after LY293111 treatment. The percentage of cells in S phase increased from 9.8 to 19.3 and 27.5% in MiaPaCa-2 cells and from 6.3 to 13.3 and 31.6% in AsPC-1 cells, respectively (Table 1). The increase in proportion of cells in the S phase was accompanied by a concomitant decease in the proportion in the G_2/M phase, but this only reached statistical significance in MiaPaCa-2 cells.

LY293111 induces cytochrome c release, and caspase-9, caspase-3 and caspase-7 activation, and also induces cleavage of poly ADP-ribose polymerase

We further investigated the molecular changes involved in LY293111-induced apoptosis in pancreatic cancer cells. After 250 nmol/l LY293111 treatment, there was a time-dependent increase in the level of cytochrome *c* in the cytosolic fraction of MiaPaCa-2 and AsPC-1 cells with a concurrent decrease in the level of cytochrome *c* in the mitochondrial fraction (Fig. 1). Released cytochrome *c* combines with a cytosolic factor, Apaf-1, to form a complex called the apoptosome, which activates caspase-9 by the hydrolysis of ATP and dATP. The inactive procaspase-9 was cleaved from its 45-kDa pro-form into 32- and 21-kDa fragments that form the active caspase-9 (Fig. 2). In contrast, we did not observe activation of caspase-8, which is involved in the death receptor-induced apoptosis through the binding of death ligands such as tumor necrosis factor-α and Fas to their receptors (Fig. 2). Treatment with LY293111 induced marked activation in caspase-3 and caspase-7. The 32-kDa

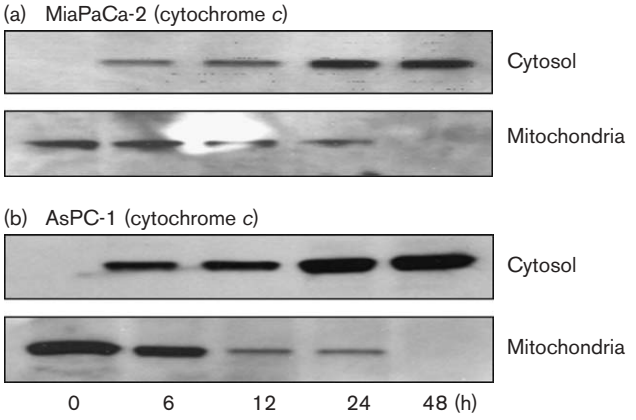
Table 1 **LY293111-induced apoptosis and S-phase arrest in MiaPaCa-2 and AsPC-1 human pancreatic cancer cells**

	Cycle phase	0 h	24 h	48 h	*F*	*P*
MiaPaCa-2	sub-G_0/G_1	1.8 ± 0.6	18.5 ± 5.8	42.8 ± 9.4	10.42	0.005
	G_0/G_1	65.8 ± 8.3	70.1 ± 7.6	69.2 ± 10.6	0.06	0.973
	S	9.8 ± 3.6	19.3 ± 2.6	27.5 ± 5.6	4.61	0.042
	G_2/M	24.4 ± 7.4	10.6 ± 4.1	3.3 ± 1.5	4.67	0.041
AsPC-1	sub G_0/G_1	0.8 ± 0.5	13.5 ± 3.4	31.4 ± 6.7	12.50	0.003
	G_0/G_1	79.4 ± 8.9	71.4 ± 7.6	62.1 ± 5.3	1.36	0.304
	S	6.3 ± 3.9	13.3 ± 4.6	31.6 ± 8.3	4.86	0.037
	G_2/M	14.3 ± 4.4	15.3 ± 3.5	6.3 ± 4.2	1.48	0.278

Table shows cell cycle distribution measured by flow cytometry in MiaPaCa-2 and AsPC-1 cells treated with LY293111 for 24 or 48 h. Data from four separate experiments were expressed as mean ± SEM of the percentage of total cells (for the sub-G_0/G_1 fraction) and then as percentage of the remaining cells (for the cell cycle phases). The *F* and *P* values from the one-way analysis of variance are also shown. There was a statistically significant increase in the proportion of cells in the sub-G_0/G_1 and S fractions following LY293111 treatment in both cell lines. The decrease in the proportion of cells in the G_2/M phase only reached statistical significance in MiaPaCa-2 cells.

inactive procaspase-3 was cleaved to produce the 17- and 11-kDa fragments that form active caspase-3 (Fig. 2). The activation of caspase-7 was shown only by the decrease in the inactive form of the procaspase-7, as the antibody does not detect the active form of caspase-7 (Fig. 2). The cleavage of PARP, which is a substrate for the activated caspase-3 and caspase-7, was also observed after LY293111 treatment. The time-dependent increase of the 85-kDa fragments from PARP cleavage was shown in both MiaPaCa-2 and AsPC-1 cells (Fig. 2).

Fig. 1



LY293111 induced cytochrome *c* release from the mitochondria into the cytosol in (a) MiaPaCa-2 and (b) AsPC-1 human pancreatic cancer cells. The cells were treated with 250 nmol/l LY293111 for 6, 12, 24 and 48 h. The mitochondrial and cytosolic fractions were separated as described previously. Proteins from each sample (30 μg) were separated on 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and were detected using monoclonal cytochrome *c* antibody. Data represent results from three separate experiments.

LY293111 decreases Bcl-2, Mcl-1 expression and increases Bax expression

LY293111-induced pancreatic cancer cell apoptosis was associated with a marked time-dependent decrease in the expression of both Bcl-2 and Mcl-1, with concomitant increase in the expression of Bax (Fig. 3). The Bax/Bcl-2 ratio was increased between 25- and 100-fold, as measured by band density after 48 h of treatment.

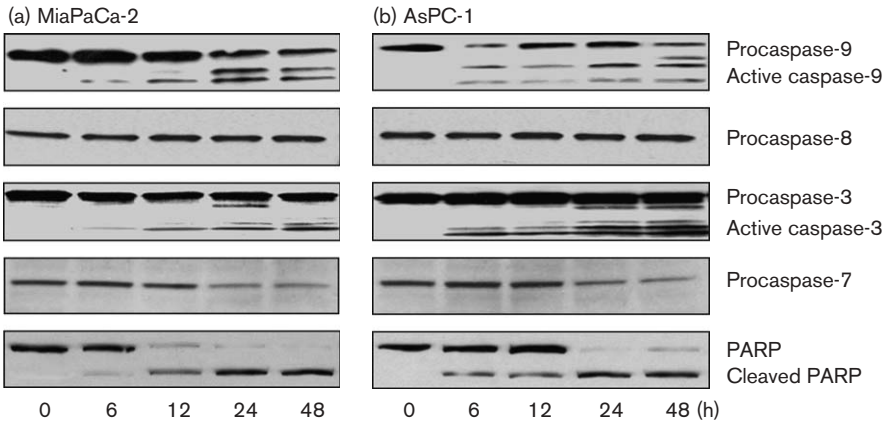
LY293111 downregulates CDK2, cyclin A and cyclin E expression

In order to determine the mechanisms involved in the distinctive S-phase arrest observed in these pancreatic cancer cells after LY29311 treatment, a number of proteins that regulate the progression of cells at G_1/S and G_2/M checkpoints were investigated. Treatment of cells with 250 nmol/l LY293111 decreased the expression of cyclin A, cyclin E and CDK2 in both MiaPaCa-2 and AsPC-1 cells (Fig. 4). Changes in concentrations of these proteins were seen at 24 h in MiaPaCa-2 cells, but occurred within 12 h in AsPC-1 cells. The expression of all three proteins was almost totally abolished after 48 h treatment with LY293111 in both cell lines. On the other hand, the expression of cyclin B1, cyclin D and CDK4 was not significantly changed by LY293111 (data not shown).

LY293111 has no effect on cyclin-dependent kinase inhibitors p21 and p27 expression

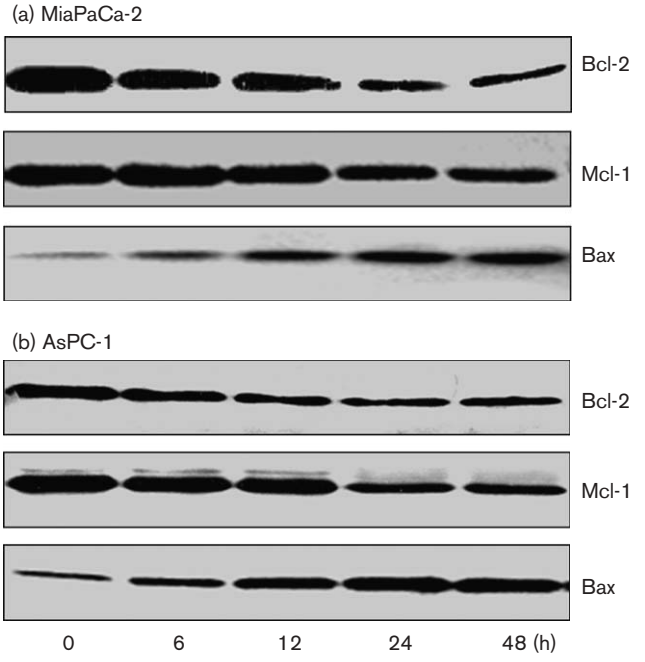
Despite the significant S-phase arrest observed in these pancreatic cancer cells after LY293111 treatment, no

Fig. 2



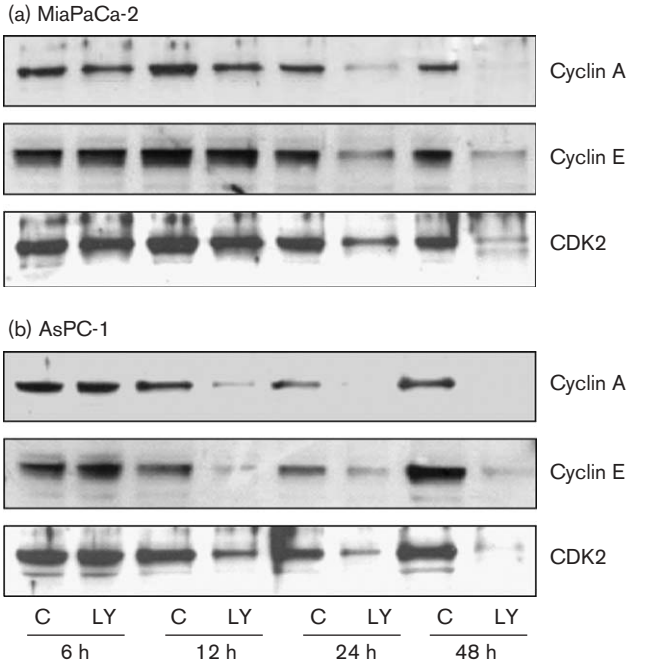
LY293111 induced time-dependent activation of caspase-9, caspase-3 and caspase-7, but not caspase-8, in (a) MiaPaCa-2 and (b) AsPC-1 human pancreatic cancer cells. LY293111 also induced poly ADP-ribose polymerase (PARP) cleavage in both cell lines. Inactive procaspase-9 was cleaved to produce 35- and 31-kDa fragments that form the active caspase-9. Inactive procaspase-3 was cleaved to produce 17- and 11-kDa fragments that form the active caspase-3. The activation of caspase-7 was represented by the decreased expression of procaspase-7 after LY293111 treatment. PARP was time dependently cleaved from 116- into 85-kDa fragments. No activation of caspase-8 was seen up to 48 h of treatment with LY293111.

Fig. 3



LY293111 reduced expression of Bcl-2 and Mcl-1, whereas it induced Bax expression in (a) MiaPaCa-2 and (b) AsPC-1 human pancreatic cancer cells. The cells were treated with 250 nmol/l LY293111 for 6, 12, 24 and 48 h, and 30 μg cell lysate proteins were separated on 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and detected using specific antibodies.

Fig. 4



LY293111 (LY) decreased cyclin A, cyclin E and CDK2 expression in (a) MiaPaCa-2 and (b) AsPC-1 human pancreatic cancer cells. The cells were treated with 250 nmol/l LY293111 for 6, 12, 24 and 48 h, and 30 μg cell lysate proteins were separated on 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The proteins were detected using cyclin A, cyclin E and CDK2 antibodies. C, control.

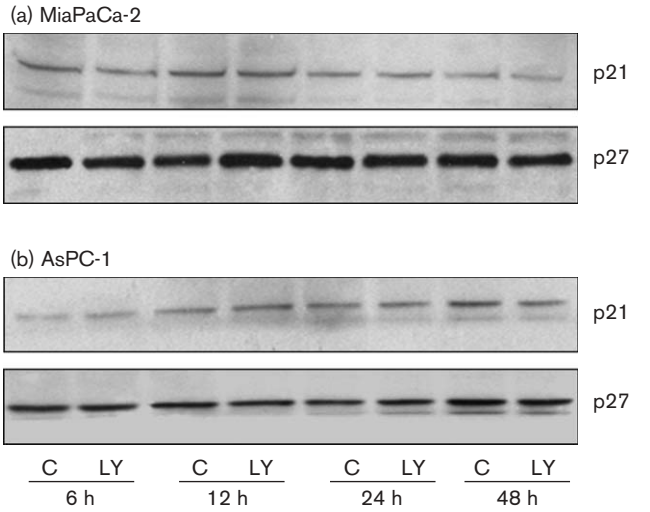
significant changes in the expression of p21 or p27 were seen up to 48 h (Fig. 5).

Discussion

The aggressive nature of the pancreatic cancer and its lack of responsiveness to conventional therapy have led clinicians and oncologists to investigate novel treatments for this devastating disease. Our previous studies have shown the importance of LOX and LTB4 metabolism in the proliferation of human pancreatic cancer, the selective LTB4 receptor antagonist LY293111 inhibits proliferation and induces apoptosis of pancreatic cancer cells both *in vitro* and *in vivo* [28,29]. In the present study, we investigated the molecular mechanisms of LY293111-induced apoptosis and cell cycle arrest to allow a better understanding of the mechanism of LY293111 as a novel treatment for human malignancies.

As expected, all the cytochrome *c* was localized to the mitochondria in control cells. In contrast, cytochrome *c* was detected in the cytosol as early as 6 h after LY293111 treatment. After 48 h, almost all the cytochrome *c* had been released from the mitochondria into the cytosol. A time-dependent activation of the initiator caspase, caspase-9 was observed after LY293111 treatment. The

Fig. 5



Effect of LY293111 (LY) treatment on expression of p21 and p27 in (a) MiaPaCa-2 and (b) AsPC-1 human pancreatic cancer cells. The cells were treated with 250 nmol/l LY293111 for 6, 12, 24 and 48 h, and 30 μg cell lysate proteins were separated on 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. No significant changes in p21 or p27 expression were seen up to 48 h after LY293111 treatment. C, control.

activation of caspase-9 was detected at 6 h and was maximal at 48 h. On the other hand, caspase-8, the initiator caspase involved in the death receptor-mediated pathway, remained in its inactive form even 48 h after treatment by LY293111. These results indicate that LY293111 induces apoptosis in human pancreatic cancer cells through the mitochondria-mediated pathway.

Downstream apoptotic signals were also studied following LY293111 treatment. LY293111 induced time-dependent activation of both caspase-3 and caspase-7. PARP, a nuclear protein implicated in DNA repair, is one of the earliest proteins targeted for a specific cleavage by the effector caspases during apoptosis. In response to treatment with LY293111, PARP was cleaved from the 116-kDa protein to the 85-kDa fragment characteristic of apoptosis.

The Bcl-2 family of proteins, which contains more that 10 members, plays an important role in regulating the apoptosis process. The expression of both Bcl-2 and Mcl-1 was markedly decreased in LY293111-induced pancreatic cancer cell apoptosis, with a concomitant increase in the Bax expression. The ratio of Bax and Bcl-2 was even dramatically increased, with a 25–100-fold increase after 48 h of treatment. A high Bax/Bcl-2 ratio has been shown to favor apoptosis.

LY293111 has previously been shown to inhibit proliferation in multiple human pancreatic cancer cells. This antiproliferative effect appears to be due to its ability to induce S-phase cell cycle arrest and apoptotic cell death. Progression of the cells from G_1 to S phase of the cell cycle is associated with the transcriptional activation by the E2F-1 transcription factor of a number of genes whose expression is required for the G_1/S-phase transition [30,31]. The cyclin A/CDK2 complex has been shown to activate E2F-1 binding and promote cell progression through the S phase and to enter the G_2/M phase of the cell cycle. Inhibition of the cyclin A/CDK2 activity is associated with S-phase arrest and possibly apoptosis of the cells [32,33]. Resveratrol and gemcitabine have been shown to induce S-phase arrest and apoptosis in various human cancers. These drugs inhibit DNA synthesis and cells are arrested in the S phase of the cell cycle with DNA stand damage. Cells arrested in the S phase are more likely to undergo apoptotic cell death through p53-dependent and p53-independent pathways [34,35]. In the present study, LY293111 significantly decreased the expression of cyclin A, cyclin E and CDK2 in pancreatic cancer cells. This corresponds with the marked S-phase arrest and apoptosis observed. Proteins involved in the early G_1/S as well as G_2/M phase transitions, such as cyclin D, cyclin B1 and CDK4, did not show significant changes following LY293111 treatment.

Inhibitors of CDK belonging to the Cip/Kip family, such as p21 and p27, act on a number of cyclin/CDK complexes, including the cyclin A, D and E-dependent kinases. P21 is induced by p53 in response to DNA damage. P21 can induce cell cycle arrest at the G_1 or S phase to allow cells to repair the damaged DNA. If the damage is too severe to be repaired, then apoptosis is initiated to remove the damaged cells [30,31]. Enhanced expression of p21 may trigger G_1-phase cell cycle arrest through the inhibition of cyclin A/CDK2 activity and induction of apoptosis in a p53-dependent pathway [36]. In our study, we did not observe significant changes in either p21 or p27 expression after LY293111 treatment. This indicates that the cell cycle arrest must be mediated by a pathway independent of these CDK inhibitors. It is likely that the decreased expression of cyclin A, cyclin E and CDK2 are involved. It should be noted that p53 is inactivated by mutations in the cell lines used in this study and so the mechanism of LY293111-induced apoptosis must be independent of p53.

In conclusion, the present studies show that LY293111-induced apoptosis in pancreatic cancer cells is through the mitochondrial pathway, with the release of cytochrome *c* and the activation of caspase-9, as well as downstream effector caspases. LY293111 reduced the expression of the antiapoptotic proteins, Bcl-2 and Mcl-1, whereas it induced expression of the proapoptotic protein, Bax. The resulting increase in the Bax/Bcl-2 favors apoptosis. LY293111 also induced S-phase cell cycle arrest in these cells with downregulation of cyclin A, cyclin E and CDK2 proteins. The findings provide mechanistic information on the effects of LY293111 in pancreatic cancer. LY293111 or similar drugs may be valuable weapons for the future fight against this devastating disease.

Acknowledgements

The selective leukotriene B4 receptor antagonist LY293111 was a gift from Drs Jake Starling and Jerome Fleisch, Lilly Research Laboratories (Indianapolis, Indiana, USA).

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