Among various molecular strategies by which prostate cancer cells evade apoptosis, phosphoinositide 3-kinase (PI3K)/Akt signaling represents a dominant survival pathway. However, different prostate cancer cell lines such as LNCaP and PC-3 display differential sensitivity to the apoptotic effect of PI3K inhibition in serum-free media, reflecting the heterogeneous nature of prostate cancer in apoptosis regulation. Whereas both cell lines are equally susceptible to LY294002-mediated Akt dephosphorylation, only LNCaP cells default to apoptosis, as evidenced by DNA fragmentation and cytochrome c release. In PC-3 cells, Akt deactivation does not lead to cytochrome c release, suggesting that the intermediary signaling pathway is short-circuited by an antiapoptotic factor. This study presents evidence that Bcl-xL overexpression provides a distinct survival mechanism that protects PC-3 cells from apoptotic signals emanating from PI3K inhibition. First, the Bcl-xL/BAD ratio in PC-3 cells is at least an order of magnitude greater than that of LNCaP cells. Second, ectopic expression of Bcl-xL protects LNCaP cells against LY294002-induced apoptosis. Third, antisense down-regulation of Bcl-xL sensitizes PC-3 cells to the apoptotic effect of LY294002. The physiological relevance of this Bcl-xL-mediated survival mechanism is further supported by the protective effect of serum on LY294002-induced cell death in LNCaP cells, which is correlated with a multifold increase in Bcl-xL expression. In contrast to Bcl-xL, Bcl-2 expression levels are similar in both cell lines, and do not respond to serum stimulation, suggesting that Bcl-2 may not play a physiological role in antagonizing apoptosis signals pertinent to BAD activation in prostate cancer cells.

The key role of the phosphoinositide 3-kinase (PI3K)/Akt signaling cascade in promoting cell survival downstream of a plethora of trophic signals has been well characterized (1–5). Activation of PI3K leads to an increase in its lipid products, phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate, in plasma membranes. These lipid second messengers facilitate the membrane co-localization of Akt with phosphoinositide-dependent kinases, leading to Akt phosphorylation and activation (6). Activated Akt, in turn, mediates antiapoptotic signaling through the phosphorylating inactivation of a multitude of downstream targets involved in apoptosis regulation, including the two key regulators of the cell death machinery BAD and procaspase 9 (4). The paradigm of this PI3K/Akt signaling cascade provides a framework to account for the ability of extracellular growth factors to promote cell survival (1–5). However, in nearly 50% of prostate tumors, this PI3K/Akt survival pathway is constitutively upregulated because of mutations of the tumor suppressor PTEN (7–10) that functions as a negative regulator of PI3K through its lipid phosphatase activity (11–13). Evidence suggests that dysregulation of the PI3K/Akt signaling cascade furnishes a mechanism whereby prostate tumor cells withstand the withdrawal of exogenous survival factors (14, 15) or androgen (16). Such a resistant phenotype is manifest in two widely used prostate cancer cell lines, androgen-dependent LNCaP and androgen-independent PC-3, both of which display enhanced Akt activation because of loss of PTEN function (11, 12). Whereas most eukaryotic cells will undergo apoptosis when deprived of exogenous trophic factors (17, 18), LNCaP and PC-3 cells survive serum deprivation for an extended period of time (19).

However, despite its up-regulated status, the degree of dependence on the PI3K/Akt pathway for evading apoptosis signals varies between these two cell lines. In serum-free media, LNCaP cells default to apoptosis when exposed to LY294002, a PI3K-specific inhibitor (14, 15). In contrast, PC-3 cells are resistant to LY294002-induced apoptosis. This dichotomy underscores the existence of a PI3K-independent survival mechanism in PC-3 cells that could counteract apoptosis signals generated by PI3K inhibition. Because LY294002 mediates apoptosis via a cytochrome c-dependent pathway in LNCaP cells (15), we hypothesized that PC-3 cells withstood PI3K/Akt inactivation by short-circuiting the downstream pathway leading to cytochrome c release. Accordingly, we turned our attention to the proapoptotic Bcl-2 family member BAD, a key downstream effector of Akt (5), and related Bcl-2 family members. It has been demonstrated that in the absence of activated Akt, BAD forms heterodimers with Bcl-xL, an antiapoptotic protein that prevents the release of cytochrome c from mitochondria (20–22). This complex formation abrogates the antiapoptotic function of Bcl-xL (23, 24), thereby facilitating apoptotic death via a cytochrome c-dependent pathway. Conversely, when Akt is activated, BAD becomes phosphorylated and translocated into the cytoplasm through binding with the phosphoserine-binding protein 14-3-3 (25, 26). The sequestration of BAD from
mitochondria frees Bcl-xL to facilitate antiapoptotic signaling. As a consequence, the dynamic interaction between Bcl-xL and BAD represents a critical determinant of cell fate downstream of the PI3K/Akt cascade, and may represent an alternative mechanism for cancer cells to evade apoptosis. In this paper, we demonstrate that Bcl-xL overexpression underlies the molecular basis for resistance to the induction of apoptosis by LY294002 in prostate cancer cells. Moreover, our data indicate that serum protects LNCaP cells from LY294002-induced apoptosis, in part, by up-regulating the expression of Bcl-xL.

MATERIALS AND METHODS

Cell Culture—To assess the effect of Bcl-xL expression levels on the sensitivity to the apoptotic effect of LY294002, androgen-responsive LNCaP (p53+/−) and androgen-nonresponsive PC-3 (p53−) human prostate cancer cells were tested. Prostate cancer cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). For individual experiments, 2.5 × 10^5 PC-3 cells and 5 × 10^5 LNCaP cells were grown in 10% FBS-supplemented RPMI 1640 medium in T-25 flasks for 1 and 2 days, respectively, followed by LY294002 treatment in serum-free RPMI medium.

Cell Viability Assay—The effect of LY294002 on cell viability was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in 96-well plates, in which 8,000 PC-3 or 10,000 LNCaP cells/well were added to triplicate wells. Cells were exposed to 25 μM LY294002 or MeSO vehicle in serum-free RPMI 1640 medium at 37 °C in 5% CO_2 for the indicated time. The medium was removed and replaced by 200 μl of a chilled hypotonic solution (220 mM mannitol, 68 mM sucrose, 50 mM Pipes-KOH (pH 7.4), 50 mM KCl, 5 mM EDTA, 2 mM MgCl_2, 1 mM dithiothreitol, and a mixture of protease inhibitors consisting of 100 μM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 80 nM aprotinin, 5 μM bestatin, 1.5 μM E-64 protease inhibitor, 2 μM leupeptin, and 1 μM pepstatin A). After a 45-min incubation on ice, the mixture was centrifuged at 600 × g for 10 min. The supernatant was collected in a microcentrifuge tube and centrifuged at 14,000 rpm for 30 min. An equivalent amount of protein (50 μg) from each supernatant was resolved in a 10% SDS-polyacrylamide gel. Bands were transferred to nitrocellulose membranes, and analyzed by immunoblotting with anti-cytochrome c antibodies, as described below.

Immunoblotting—The sample preparation procedure for Akt analysis differed from that of other cellular proteins, which are elaborated as follows. For Akt sample preparation, cells in T-75 flasks were collected by scraping, and suspended in 60 μl of phosphate-buffered saline. Two μl of the suspension was taken for protein analysis using the Bradford assay kit (Bio-Rad). To the remaining solution was added the same volume of 2× SDS-PAGE sample loading buffer (100 μM Tris-HCl, pH 6.8, 4% SDS, 5% β-mercaptoethanol, 20% glycerol, and 0.1% bromophenol blue). The mixture was sonicated briefly, and boiled for 5 min. Equal amounts of proteins were loaded onto 10% SDS-PAGE gels. For other cellular proteins, the cells were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Mouse monoclonal anti-cytochrome c was from BD Pharmingen. Mouse monoclonal anti-actin was from ICN Biomedicals Inc. (Costa Mesa, CA).

Effect of Serum on LY294002-induced Apoptosis in LNCaP Cells—LNCaP cells (2.5 × 10^5) were cultured in 10% FBS-supplemented RPMI 1640 medium for 2 days. The cells were treated with 50 μM LY294002 or MeSO vehicle, and cultured in serum-free RPMI 1640 medium for 1 day. These cells were exposed to 10% FBS-supplemented RPMI 1640 medium with or without LY294002 (25 μM). The treated cells were collected at different time intervals for apoptosis and Western blot analyzes.

RESULTS

LNCaP and PC-3 Cells Display Differential Susceptibility to PI3K Inhibition-induced Apoptosis—The role of PI3K as a dominant mediator of survival in LNCaP cells has been well-documented.
Exposure of LNCaP cells to its specific inhibitor LY294002 (25 μM) in serum-free medium resulted in rapid and complete loss of phospho-Akt. Western blot analysis indicates that Akt was completely dephosphorylated within the first hour of drug treatment, whereas the level of phospho-Akt remained unchanged in Me2SO vehicle-treated cells throughout the 24-h period (Fig. 1A). These data confirm the obligatory role of PI3K lipid products in maintaining the active status of Akt. This Akt deactivation was accompanied by apoptotic cell death, as characterized by morphological changes, cell viability, DNA fragmentation, and cytochrome c release from mitochondria (Fig. 1, B–E).

In PC-3 cells, the effect of LY294002 on phospho-Akt was more pronounced. Exposure of PC-3 cells to LY294002 led to a rapid disappearance of phospho-Akt in a manner similar to that in LNCaP cells. The formation of nucleosomes was measured quantitatively by Cell Death Detection ELISA with lysates equivalent to 5 × 10^5 cells for each assay. Data are the mean ± S.D. (n = 3). E, a time-dependent effect of LY294002 on cytochrome c release in PC-3 cells. The experimental conditions were identical to that described in the legend to Fig. 1, except that PC-3 cells were used. A, a time-dependent effect of LY294002 (25 μM) on the phosphorylation state of Akt. Exposure of PC-3 cells to LY294002 led to a rapid disappearance of phospho-Akt in a manner similar to that in LNCaP cells. B, morphology of PC-3 cells treated with Me2SO vehicle (left panel) or 25 μM LY294002 (right panel) in serum-free RPMI 1640 medium for 24 h. C, time-dependent effect of LY294002 (25 μM) on the viability of PC-3 cells. PC-3 cells (8,000 cells/well) were exposed to 25 μM LY294002 (closed circle) or Me2SO vehicle (open circle) for the indicated times. Viable cells were examined by the MTT assay. Data are the mean ± S.D. (n = 6). E, a time-dependent effect of LY294002 on cytochrome c release in PC-3 cells.

Cytosolic-specific, mitochondria-free lysates were prepared. An equivalent amount of protein (50 μg) from individual lysates was electrophoresed, and probed by Western blot with anti-cytochrome c antibodies.

FIG. 2. PC-3 cells are susceptible to LY294002-induced Akt dephosphorylation, but resist the induction of apoptosis by preventing cytochrome c release. The experimental conditions were identical to that described in the legend to Fig. 1, except that PC-3 cells were used. A, a time-dependent effect of LY294002 (25 μM) on the phosphorylation state of Akt. Exposure of PC-3 cells to LY294002 led to a rapid disappearance of phospho-Akt in a manner similar to that in LNCaP cells. B, morphology of PC-3 cells treated with Me2SO vehicle (left panel) or 25 μM LY294002 (right panel) in serum-free RPMI 1640 medium for 24 h. C, time-dependent effect of LY294002 (25 μM) on the viability of PC-3 cells. PC-3 cells (8,000 cells/well) were exposed to 25 μM LY294002 (closed circle) or Me2SO vehicle (open circle) for the indicated times. Viable cells were examined by the MTT assay. Data are the mean ± S.D. (n = 3). D, time course of the formation of nucleosomal DNA in PC-3 cells treated with Me2SO vehicle (open bars) or 25 μM LY294002 (gray bars). Data are the mean ± S.D. (n = 3). E, a time-dependent effect of LY294002 on cytochrome c release in PC-3 cells.
Fig. 3. LY294002 treatment facilitates the dephosphorylation and targeting of BAD to mitochondria in LNCaP and PC-3 cells. Cells were treated with Me2SO vehicle (−) or 25 μM LY294002 (+) in serum-free RPMI 1640 medium for 12 h, and lysed. The top panels show the decrease in phospho-Ser136-BAD in cell lysates in response to LY294002 treatment, whereas there was no effect on the expression level of BAD. The middle and bottom panels illustrate the mitochondrial targeting of BAD. The cytoplasmic and mitochondrial fractions were isolated, electrophoresed, and probed by Western blot with rabbit anti-BAD antibodies. Actin and cytochrome c oxidase were isolated, electrophoresed, and probed by Western blot with rabbit anti-FLAG antibodies. Bcl-2, and BAD with PC-3 and LNCaP cells by Western blot analysis. B, ascending expression levels of ectopic Bcl-xL in B11, B1, and B3 clones. The band for ectopic Bcl-xL contained a FLAG tag (8 amino acids long) from the construct, thus migrating slower than endogenous Bcl-xL.

Fig. 4. A, comparison between the basal expression levels of Bcl-xL, Bcl-2, and BAD with PC-3 and LNCaP cells by Western blot analysis. B, ascending expression levels of ectopic Bcl-xL in B11, B1, and B3 clones. The band for ectopic Bcl-xL contained a FLAG tag (8 amino acids long) from the construct, thus migrating slower than endogenous Bcl-xL.

Fig. 5. Ectopic Bcl-xL protects LNCaP cells from LY294002-induced apoptosis by attenuating cytochrome c release in an expression level-dependent manner. A, formation of nucleosomal DNA in LNCaP (LN) cells and B1, B1, and B3 clones treated with Me2SO (open bars) or 25 μM LY294002 (gray bars) at 12 (left panel) and 24 h (right panel). The formation of nucleosomes was quantitatively measured by Cell Death Detection ELISA with lysates equivalent to 5 × 10⁵ cells for each assay. Data are the mean ± S.D. (n = 3). B, effect of LY294002 on cytochrome c release in LNCaP cells and the three Bcl-xL overexpressing clones at 24 h. Cells were treated with Me2SO vehicle (−) or 25 μM LY294002 (+) for 24 h. Cytosolic-specific, mitochondria-free lysates were prepared. An equivalent amount of protein (50 μg) from individual lysates was electrophoresed, and probed by Western blot with anti-cytochrome c antibodies.

Bcl-xL Mediates a PI3K-independent Survival Pathway

Phosphorylation and mitochondrial translocation of BAD in LY294002-treated LNCaP and PC-3 cells is demonstrated in Fig. 3. As shown, Western blot analysis indicates that exposure to the PI3K inhibitor led to a decrease in phospho-Ser136-BAD in cell lysates while there was no effect on the expression level of BAD (top panels). Mitochondrial targeting is demonstrated by a decrease in total levels of BAD protein in the cytoplasm accompanied by a concurrent increase in mitochondria in both cell lines (middle and bottom panels). Together, these findings suggest that PC-3 cells were able to evade PI3K/Akt inhibition-mediated apoptosis by short circuiting the intermediary signaling between the BAD activation and cytochrome c release via a yet unresolved mechanism.

Bcl-xL Overexpression and Apoptosis Resistance in PC-3 Cells—To shed light onto the mechanistic basis underlying this differential response to BAD activation, we examined the expression of BAD, Bcl-xL, and a related Bcl-2 family member, Bcl-2, in LNCaP and PC-3 cells. Western blot analysis indicates that these two cell lines displayed a distinct difference in Bcl-xL expression levels, whereas those of BAD were virtually identical between them (Fig. 4A). As a consequence, the ratio of Bcl-xL to BAD in PC-3 cells was at least an order of magnitude higher than that in LNCaP cells, which is consistent with the finding reported in the literature (29). In contrast, the expression level of Bcl-2 was moderately lower in PC-3 cells than in LNCaP cells, excluding the involvement of Bcl-2 in the resistance to LY294002-induced cell death in PC-3 cells. In view of the antiapoptotic effect of Bcl-xL, we hypothesized that Bcl-xL overexpression in PC-3 cells conferred protection against apoptotic signals generated from PI3K inhibition. Accordingly, we assessed the effect of enforced Bcl-xL expression in LNCaP cells on LY294002-induced apoptosis.
Ectopic Bcl-xL Expression Protects LNCaP Cells from LY-294002-induced Apoptosis—LNCaP cells were transfected with the G418-selectable Bcl-xL expression construct pSFFV-Neo/Bcl-xL-FLAG. Three transfected clones (B11, B1, and B3), which displayed ascending expression levels of ectopic Bcl-xL protein (Fig. 4B), were isolated for testing. The expression levels of ectopic Bcl-xL in B11, B1, and B3 were ~20, 150, and 500%, respectively, of that of the endogenous counterpart in PC-3 cells. Among these three clones, B3 cells displayed decreased endogenous Bcl-2 expression, whereas in the other two clones remained relatively unaltered in comparison to untransfected LNCaP cells. These three transfected Bcl-xL clones were used to examine the impact of the Bcl-xL expression level on susceptibility to LY294002-induced apoptosis vis à vis parental LNCaP cells.

Fig. 5A depicts a dose-dependent protective effect of ectopic Bcl-xL on LY294002-mediated cell death at 12 (left panel) and 24 h (right panel). The extent of cytoprotection correlated with the Bcl-xL expression level among the three Bcl-xL clones. In line with the data obtained with PC-3 cells, this differential resistance was attributable to the ability of Bcl-xL to suppress cytochrome c release into the cytoplasm (Fig. 5B). As demonstrated in B3 cells, the high level of ectopic Bcl-xL expression completely blocked the release of cytochrome c following LY294002 treatment, thereby rendering the antiapoptotic phenotype.

As part of the control, because PC-3 cells differ from LNCaP cells in functional p53 functional status, (p53−/− versus p53+/+, respectively), we also examined the effect of p53 overexpression in PC-3 cells on LY294002-induced apoptosis. However, p53-overexpressing PC-3 cells were equally resistant to LY294002 as the parent cells (data not shown), excluding the possible involvement of p53 in sensitizing prostate cancer cells to PI3K inhibition-induced apoptosis.

Antisense Down-regulation of Bcl-xL Reduces the Threshold of LY294002-mediated Apoptosis in PC-3 Cells—We further investigated the effect of Bcl-xL on cellular sensitivity to LY294003 by using an antisense oligonucleotide strategy to down-regulate Bcl-xL in PC-3 cells. A phosphothioate oligonucleotide (27) was used to attenuate the expression of Bcl-xL in PC-3 cells with an oligonucleotide with a mismatched sequence (CGACACGTACCTCTCGCATT) (29) as control. As shown in Fig. 6A, this antisense oligonucleotide decreased the level of Bcl-xL expression in a dose-dependent manner, whereas no significant change was noted with the mismatched oligonucleotide at 1 μM. At high doses (≥2 μM), this antisense oligonucleotide was cytotoxic, possibly as a result of Bcl-xL ablation. Nevertheless, at 1 μM, it could reduce the Bcl-xL expression to a level comparable with that of LNCaP cells without causing significant cell death.

Treatment with this nontoxic level of antisense oligonucleotide increased the chemosensitivity of PC-3 cells to LY294002, which induced DNA fragmentation within 2 h of
treatment, whereas PC-3 cells treated with the mismatch oligonucleotide (1 μM) remained unaffected by PI3K inhibition (Fig. 6B). This discrepancy in response to LY294002 was correlated with the respective abilities to maintain the mitochondrial integrity (Fig. 6C). As shown, exposure of PC-3 cells transfected with the antisense oligonucleotide to LY294002 led to increased cytochrome c release, whereas no increase in cytosolic cytochrome c was observed with the mismatch oligonucleotide-treated cells.

Together, these data suggest that basal Bcl-xL expression underlies the discrepancy between LNCaP and PC-3 cells in the sensitivity to the apoptotic effect of PI3K inhibition. Conceivably, PI3K/Akt signaling and Bcl-xL represent two distinct mechanisms at different cellular levels that cancer cells use to enhance apoptosis threshold in the face of growth factor stimulation. The relative roles of these two mechanisms in cytoprotection, however, remained unclear when both pathways were stimulated. To address this issue, we examined the protective effect of serum on LY294002-induced apoptosis in LNCaP cells, of which the mechanism has yet been resolved (15).

The Protective Effect of Serum on LY294002-mediated Apoptosis Is Correlated with Increased Bcl-xL Expression—To assess the effect of serum, LNCaP cells were exposed to serum-deprived RPMI 1640 medium for 24 h before supplementing the medium with 10% FBS. The extent of apoptotic cell death induced by LY294002 in the presence of 10% FBS was significantly less than that without serum at 12 and 24 h (p < 0.05) (Fig. 7A), which is in line with the data previously reported (15). Western blot analysis indicates that exposure to serum induces a multifold increase in both Akt phosphorylation and Bcl-xL expression in comparison to basal levels (Fig. 7B). Bcl-2 expression levels, however, did not respond to serum stimulation, and remained relatively unaltered throughout the course of this investigation. Exposure of LNCaP cells to LY294002 in 10% FBS-supplemented medium led to rapid dephosphorylation of Akt in a manner similar to that in the absence of serum. Meanwhile, Bcl-xL expression levels remained up-regulated under the same conditions, suggesting its role in the effect of serum on apoptosis resistance.

DISCUSSION
Prostate tumor cells acquire multiple molecular strategies to evade apoptosis in the course of progression to advanced states (19). Among various survival tactics utilized by prostate cancer cells, the up-regulation of PI3K/Akt signaling through PTEN mutation or constitutive activation of growth factor receptors is especially noteworthy (11, 12). By elevating the level of Akt activation, cancer cells are able to raise the apoptosis threshold in response to trophic factor withdrawal or cytokine exposure. Consequently, targeting the survival pathway mediated by PI3K/Akt signaling is widely considered a viable approach for cancer therapy. Nevertheless, different prostate tumor cell lines display differential susceptibility to the apoptotic effect of PI3K inhibition, reflecting the heterogeneous nature of prostate cancer in apoptosis regulation. Our study indicates that PC-3 and LNCaP cells were equally susceptible to LY294002-mediated dephosphorylation of Akt, but differed in the consequent effect on cytochrome c release from mitochondria. These data suggest that the effect of Akt inactivation on cytochrome c release through BAD activation was abrogated by an anti-apoptotic mechanism at the mitochondrial level in PC-3 cells. Because the Bcl-2 family provides a crucial link between the Akt activity and the status of mitochondrial integrity (24), we investigated the involvement of BAD and its closely related Bcl-2 members Bcl-xL and Bcl-2.

The present study presents several lines of evidence that Bcl-xL overexpression provides a distinctive survival mechanism that protects PC-3 cells from apoptosis signals emanating from PI3K inhibition. First, the Bcl-xL to BAD ratio in PC-3 cells was at least an order of magnitude greater than that of LNCaP cells. Second, ectopic expression of Bcl-xL protected LNCaP cells against LY294002-induced apoptotic death in an expression level-dependent manner. Third, antisense down-regulation of Bcl-xL sensitized PC-3 cells to the apoptotic effect of LY294002. Fourth, the physiological relevance of this Bcl-xL-mediated survival mechanism was further underscored by the protective effect of serum on LY294002-induced cell death in LNCaP cells. In the literature, Bcl-xL overexpression has also been identified as a major mediator in the resistance of PC-3 cells against apoptosis induced by the PKC inhibitor staurosporine (29). Conceivably, BAD activation represents the convergence point for apoptotic signals generated by the inhibition of PI3K and PKC. Higher levels of Bcl-xL expression in PC-3 cells are able to antagonize the proapoptotic effect of BAD, thereby conferring resistance to these apoptosis-inducing agents. Moreover, it has been reported that increased Bcl-xL expression could desensitize LNCaP and PC-3 cells to various cytotoxic agents such as paclitaxel, vinblastine, etoposide, and carboplatin (27). These data further highlight the important role of Bcl-xL expression in modulating the apoptosis threshold to either molecularly targeted or cytotoxic therapeutic agents.

Another crucial issue that warrants discussion is the physiological role of Bcl-2 vis-à-vis Bcl-xL in the cytoprotection against BAD activation because these two antiapoptotic proteins mediate a similar function in maintaining mitochondrial integrity. In addition, enforced expression of Bcl-2 has been reported to protect LNCaP cells against apoptosis induction by C2-ceramide or androgen depletion (30, 31). The present study, however, refutes the involvement of basal Bcl-2 expression in the protection of PC-3 or serum-treated LNCaP cells against LY294002-mediated apoptosis. Similar findings have also been reported with regard to the role of Bcl-2 in chemoresistance to various apoptosis-inducing agents in PC-3 cells (27).

This novel survival pathway bears biochemical relevance to cancer therapy. Recent advances in the understanding of signal transduction in cancer cells have led to the development of therapeutic agents targeting growth factor receptor tyrosine kinases (32, 33), some of which involve PI3K/Akt signaling as a downstream effector. Levels of Bcl-xL expression may affect the efficacy of these tyrosine kinase inhibitors in prostate cancer cells. Moreover, inhibition of PI3K/Akt signaling has been reported to sensitize cancer cells to apoptosis induced by cytotoxic agents such as doxorubicin and paclitaxel (34–36), or cytokines (37). Conceivably, the sensitizing effect of PI3K/Akt inhibition to apoptotic signals may be antagonized by enhanced Bcl-xL expression in advanced prostate cancer cells. Finally, as demonstrated by the mechanism of serum-induced protection against LY294002-induced apoptosis, modulation of the expression level of Bcl-xL may represent an important strategy to optimize the efficacy of chemotherapeutic agents, which is currently under investigation in this laboratory.

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