Galectin-3 Inhibits Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand–Induced Apoptosis by Activating Akt in Human Bladder Carcinoma Cells

Natsuo Oka, 1,2 Susumu Nakahara, 1,3 Yukinori Takenaka, 3 Tomoharu Fukumori, 2 Victor Hogan, 1 Hiro-omi Kanayama, 2 Takashi Yanagawa, 1,4 and Avraham Raz 1

1Tumor Progression and Metastasis, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan; 2Department of Urology, The University of Tokushima School of Medicine, Tokushima, Japan; 3Department of Otolaryngology and Sensory Organ Surgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; and 4Department of Orthopedic Surgery, Faculty of Medicine, Gunma University, Gunma, Japan

Abstract
The antiapoptotic molecule galectin-3 was previously shown to regulate CD95, a member of the tumor necrosis factor (TNF) family of proteins in the apoptotic signaling pathway. Here, we question the generality of the phenomenon by studying a different member of this family of proteins [e.g., TNF-related apoptosis-inducing ligand (TRAIL)], which induces apoptosis in a wide variety of cancer cells. Overexpression of galectin-3 in J82 human bladder carcinoma cells rendered them resistant to TRAIL-induced apoptosis, whereas phosphatidylinositol 3-kinase (PI3K) inhibitors (wortmannin and LY-294002) blocked the galectin-3 protecting effect. Because Akt is a major downstream PI3K target reported to play a role in TRAIL-induced apoptosis, we questioned the possible relationship between galectin-3 and Akt. Parental J82 and the control vector–transfected J82 cells (barely detectable galectin-3) exhibit low level of constitutively active Akt, resulting in sensitivity to TRAIL. On the other hand, J82 cells overexpressing galectin-3 cells expressed a high level of constitutively active Akt and were resistant to TRAIL. Moreover, the blockage of TRAIL-induced apoptosis in J82 cells seemed to be mediated by Akt through the inhibition of BID cleavage. These results suggest that galectin-3 involves Akt as a modulator molecule in protecting bladder carcinoma cells from TRAIL-induced apoptosis. (Cancer Res 2005; 65(17): 7546-53)

Introduction
Galecints are a family of carbohydrate-binding proteins characterized by a conserved amino acid sequence defined by structural similarities in their carbohydrate-binding domain and affinity for β-galactoside–containing glycoconjugates (1–3). In the last decade, it was reported that galecints play a significant role in apoptosis, several of which, such as galecint-1 and galecint-9, were implicated in the promotion of apoptosis of immune and melanoma cells, respectively (4, 5), whereas galecint-7 induces apoptosis of colon cancer cells (6). In contrast, galecint-3 was reported to be an antiapoptotic molecule (7–11), inhibiting Fas-induced T-cell apoptosis (10) as well as epithelial cell apoptosis induced by staurosporine, cisplatin, geneine, and anoikis (7–9, 11). The antiapoptotic activity of galecint-3 was also shown in galecint-3–deficient mice whereby peritoneal macrophages from galecint-3–null mice were more sensitive to apoptotic stimuli than were those from control mice (12), and galecint-3 expression in epithelial cells endowed them with resistance to apoptotic insult (7–11). The ability of galecint-3 to protect cells against agents working through different mechanisms to induce apoptosis suggests that galecint-3 regulates a common apoptosis commitment step. Galecint-3 contains the anti-death Asp-Trp-Gly-Arg (NWGR) motif that is conserved in the Bcl-2 homology domain (BH1) of the Bcl-2 family (7, 13) and determines, in part, the CD95-induced apoptotic signaling pathway in type I and type II cells (14).

Tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL), also known as Apo-2 ligand, is a proapoptotic cytokine in a family of ligands that transduce death signals through death domain–containing receptors (15–17). TRAIL is a transmembrane protein that functions by binding to two closely related receptors, DR4 and DR5 (18), which leads to the cleavage and activation of caspase-8, resulting in BID cleavage, a Bcl-2 inhibitory protein, triggering mitochondrial depolarization (19). Despite the ubiquitous expression of TRAIL receptors, some cancer cells show either partial or complete resistance to the proapoptotic effects of TRAIL for unknown reasons. Antiapoptotic molecules such as Bcl-2 and Bcl-XL may be specifically associated with resistance to TRAIL-mediated apoptosis (13). FLICE-like inhibitory protein has been shown to bind to caspase-8 and prevent the activation of downstream events leading to apoptosis, including TRAIL-mediated apoptosis (20, 21).

It is highly likely that intracellular inhibitors acting downstream of TRAIL receptors render some cells insensitive to TRAIL apoptotic stimuli because the resistance of many types of cancer cells to TRAIL can be reversed by treatment with protein synthesis inhibitors (20, 22) or chemotherapeutic agents (23). The study of the intracellular mechanisms that control TRAIL resistance has revealed the role of surviving factors like Akt that contribute resistance to apoptotic signals (24). Akt is a Ser/Thr protein kinase implicated in mediating a variety of biological responses, which include the inhibition of apoptosis and the stimulation of cellular growth. It was found to be overexpressed in some gastric adenocarcinoma and in breast, ovarian, prostate, and pancreatic cancers (25, 26). Activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway generates phosphatidylinositol-3,4,5-triphosphate, which in turn binds to the pleckstrin homology domain of Ser/Thr kinase Akt, resulting in recruitment of Akt to the cell membrane. Once activated, Akt phosphorylates specific targets such as Bad, caspase-9, and transcription factor FKHL1, thus promoting cell survival and blocking apoptosis (24).
Thus, based on the above, we questioned the role of galectin-3 in TRAIL-induced apoptosis in human epithelial bladder carcinoma cells and reported that it inhibits TRAIL-induced apoptosis by elevating Akt activity.

Materials and Methods

Antibodies and reagents. Antibodies were obtained from the following sources: anti-galectin-3 monoclonal antibody (TIB166; American Type Culture Collection (ATCC); Manassas, VA); anti-BID, anti-DR4, anti-DR5, and anti-β-actin antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); anti-phospho-Akt antibody (Ser-473) and anti-Akt antibody (Cell Signaling Technology, Beverly, MA). Pharmacologic inhibitors AG-1478, PD-98059, and SB-203580 were purchased from Calbiochem (La Jolla, CA). Wortmannin, LY-294002, and TRAIL were purchased from Sigma-Aldrich (St. Louis, MO). Lipofectamine was purchased from Invitrogen Life Technologies (Carlsbad, CA). JC-1 dye was purchased from Molecular Probes, Inc. (Eugene, OR).

Cell culture. The human bladder carcinoma cell line J82 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were grown as monolayers on plastic tissue culture dishes containing DMEM supplemented with 10% heat-inactivated fetal bovine serum and antibiotics and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO2.

Cell transfection. pBK-CMV (Stratagene, La Jolla, CA), a mammalian expression vector containing wild-type galectin-3, was constructed as previously described (11). Briefly, galectin-3 CDNA was excised from pGEM (+) with EcoRI and inserted into the vector. The proper orientation of the insert was confirmed by DNA sequencing. Each purified plasmid DNA was transfected into J82 using Lipofectamine (Invitrogen, Carlsbad, CA). After 48 hours, 800 μg/mL G418 (Invitrogen) was added to the culture for 14 days to obtain stable transfected clones. For further experiments, J82 parental, J82/V for the control vector-transfected cells, and J82/WT1, J82/WT2, and J82/WT3 for the wild-type galectin-3–transfected cells were employed. These transfected cells were maintained in complete DMEM containing 400 μg/mL G418 sulfate.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenylenetrazolium bromide assay. Cells (1 x 10³ in 100 μL of culture medium/well) were seeded into 96-well flat-bottomed plates, treated with or without drugs, and incubated for various times at 37°C in a humidified atmosphere of 95% air and 5% CO2. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylenetrazolium bromide (MTT) assay. Ten microliters of 5 mg/mL MTT solution (Sigma) were added to each well. After 3 hours of incubation, 200 μL of DMEM (Fishier Biotech, Fairlawn, NJ) were added to each well. The absorbance of each well was measured at 495 and 650 nm using a spectrophotometer (Spectra Maxi Germini, Molecular Devices, Menlo Park, CA). 7-Amino-4-methylcoumarin fluorescence plate reader (Molecular Devices, Menlo Park, CA).

Caspase assay. Caspase activity was measured using fluorogenic caspase substrates. Briefly, cells were harvested after incubation with various inhibitors (AG-1478, PD-98059, wortmannin, LY-294002, and SB-203580) in the presence or absence of TRAIL and then lysed with cell extract buffer (0.03% Nonidet P-40, 20 mmol/L HEPES (pH 7.5), 1.5 mmol/L MgCl2, 10 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L EGTA, and 1 mmol/L DTT) containing 25 μmol/L Leupeptin, 2 mmol/L Aprotinin, and 1 mmol/L Sodium orthovanadate. The lysates were centrifuged at 15,000 g for 10 minutes, and 50 μL of the cytosolic fraction was incubated for 60 minutes at 37°C in a total volume of 200 μL of caspase buffer [10 mmol/L HEPES (pH 7.5), 30 mmol/L NaCl, 2.5 mmol/L DTT] containing 25 μmol/L Ac-DEVD-AMC, Ac-IETD-AMC, or Ac-LEHD-AMC (Bachem, King of Prussia, PA). 7-Amino-4-methylcoumarin fluorescence, released by caspase activity, was measured at 405 nm using a Spectra Maxi Germini fluorescence plate reader (Molecular Devices, Menlo Park, CA).

Mitochondrial energization. Retention of JC-1 staining (Molecular Probes) was used as a measure of mitochondrial energization. This dye, existing as a monomer in solution emitting a green fluorescence, can assume a dimeric configuration emitting red fluorescence in a reaction driven by the mitochondrial transmembrane potential. Cells (1 x 10³ in 100 μL of culture medium/well) were seeded and treated with drugs and incubated for various times. JC-1 (5 μg/mL) was added during the last 30 minutes of treatment. Cells were washed twice with HBSS (in vetro) to remove unbound dye. The concentration of retained JC-1 dye was measured (490 nm excitation/600 nm emission) by a Spectra Maxi Germini fluorescence plate reader (Molecular Devices, Menlo Park, CA).

Results

Galectin-3 inhibits TRAIL-induced apoptosis. Parental J82 cells were chosen for this study as they express extremely low levels of galectin-3 (Fig. 1A, P). The cells were thus transfected with either control vector (Fig. 1A, V) or the vector containing galectin-3 cDNA (Fig. 1A, Gal-3; ref. 5). The expression level of galectin-3 in galectin-3–transfected cells was 4.8-fold higher than the controls as determined by densitometric tracing (Fig. 1A). The cytotoxic effect of TRAIL was next tested, and as can be seen from Fig. 1B and C, both parental J82 and J82/V cells (galectin-3 low expressing) were highly sensitive to TRAIL in a dose- and time-dependent manner, whereas cells overexpressing galectin-3 were refractory to TRAIL (Fig. 1B and C; ref. 6). We have also evaluated the sub-G1 fraction of propidium iodide–labeled cells by flow cytometry and confirmed that TRAIL-induced death of the control cells was due to apoptosis and that overexpression of galectin-3 inhibits it (data not shown).

Next, we investigated which signaling pathway is mainly involved in this galectin-3–mediated TRAIL resistance to apoptosis by using the mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor (PD-98059), the PI3K inhibitors (wortmannin and LY-294002), the ErB1 inhibitor (AG-1478), and the p38 inhibitor (SB-203580).

All these cells were pretreated with PD-98059 (20 μmol/L), wortmannin (1 μmol/L), LY-294002 (25 μmol/L), AG-1478 (20 μmol/L), and SB-203580 (20 μmol/L) for 4 hours, followed by treatment with TRAIL (100 ng/mL) for 24 hours, and apoptosis was assessed (Fig. 2).

TRAIL alone induced apoptosis in J82 and J82/V cells, but J82/G1, J82/G2, and J82/G3 cells were resistant to apoptosis. Only wortmannin and LY-294002 pretreatment further enhanced the effects of TRAIL on apoptosis in J82 cells (by 1.5- and 1.5-fold, respectively) and J82/V cells (by 1.5- and 1.6-fold, respectively), but not significantly. Interestingly, on the other hand, pretreatment of J82/G1, J82/G2, and J82/G3 cells with only wortmannin and LY-294002 dramatically increased apoptosis when combined with TRAIL (G1: by 4.1- and 4.4-fold, respectively, P < 0.05; G2: by 4.9- and 5.7-fold, respectively, P < 0.05; G3: by 5.3- and 5.3-fold, respectively, P < 0.05), whereas PD-98059, AG-1478, and SB-203580 did not sensitize these cells to TRAIL.
Taken together, these results suggest that galectin-3-overexpressing J82 cells exert their resistance to TRAIL via the PI3K/Akt pathway.

Tumor necrosis factor–related apoptosis-inducing ligand–induced activation of caspase-3 and caspase-9 in J82 cells but not in galectin-3–overexpressing J82 cells. It is well known that TRAIL-induced apoptosis is mediated through a caspase cascade. To examine whether the PI3K/Akt pathway affects TRAIL-induced caspase activation in these cells, we examined the activation of caspase-3 and caspase-9 in cells treated with TRAIL in the presence or absence of wortmannin or LY-294002 (Fig. 3A and B). Treatment of cells with wortmannin or LY-294002 had no effect on caspase-3 and caspase-9 activity. Caspase-3 and caspase-9 were activated by TRAIL in J82 and J82/V cells, but not in J82/G1, J82/G2, and J82/G3 cells. When combined with wortmannin or LY-294002, the increase in TRAIL-induced activation of caspase-3 and caspase-9 was not significant in J82 and J82/V cells, whereas in J82/G1, J82/G2, and J82/G3 cells, TRAIL significantly enhanced the activation of both caspase-3 (G1: by 8.4- and 9.5-fold, respectively, P < 0.05; G2: by 7.1- and 7.3-fold, respectively, P < 0.05; G3: by 8.5- and 9.1-fold, respectively, P < 0.05) and caspase-9 (G1: by 7.6- and 8.2-fold, respectively, P < 0.05; G2: by 7.6- and 7.6-fold, respectively, P < 0.05; G3: by 7.7- and 8.2-fold, respectively, P < 0.05).

Previous reports have shown that death-inducing signaling complex formation is essential not only for TRAIL-induced signaling but also for activation of caspase-8 (22). To exclude the possibility of defects in caspase-8, we examined the caspase-8 activity in J82 cells and compared it with J82/G1, J82/G2, and J82/G3 cells (Fig. 3C). No difference in TRAIL-induced caspase-8 activity was seen among these cells. Pretreatment of cells with wortmannin or LY-294002 did not affect TRAIL-induced caspase-8 activity. These results suggest that resistance of J82/G1, J82/G2, and J82/G3 cells to TRAIL is not due to defects in caspase-8 activation and the PI3K/Akt pathway does not affect caspase-8 activation.

Attenuation of tumor necrosis factor–related apoptosis-inducing ligand–induced drop in mitochondrial membrane potential in galectin-3–overexpressing J82 cells. Mitochondria play an important role in apoptosis (19, 28). Both mitochondrial depolarization and the loss of cytochrome c from the mitochondrial intermembrane space have been proposed as the early events during apoptotic cell death (29). Therefore, we measured the mitochondrial membrane potential (ΔΨm) using the mitochondria-specific dye JC-1. We investigated mitochondrial dysfunction by measuring ΔΨm (Fig. 4A; ref. 30). In J82/G2 cells, no significant effect on ΔΨm was seen when incubated with TRAIL, wortmannin, or LY-294002 alone. However, combination of TRAIL with wortmannin or LY-294002 induced a significant decrease (P < 0.05) in ΔΨm (Fig. 4A, top). Similar results were obtained when J82/G1 and J82/G3 cells were used (data not shown).

In contrast, the decrease of ΔΨm in J82/V cells as well as J82 parental cells (data not shown) when treated with TRAIL alone, TRAIL plus LY-294002, or TRAIL plus wortmannin was not statistically significant (Fig. 4A, bottom). Thus, taken together, these results showed that in galectin-3–overexpressing J82 cells, the activation of the PI3K/Akt pathway inhibits loss of the mitochondrial membrane potential, resulting in inhibition of caspase-9 and caspase-3 activation.

Tumor necrosis factor–related apoptosis-inducing ligand–induced apoptosis signaling in galectin-3–overexpressing J82 cells is blocked at the level of BID cleavage. It has been reported that activation of caspase-8 by TRAIL may cleave BID (a Bcl-2 inhibitory protein) of which cleavage product triggers mitochondrial depolarization and subsequent release of cytochrome c from mitochondria (31, 32). Therefore, we next examined the effects of TRAIL on BID cleavage in these cells. Treatment of J82/G2 cells with LY-294002 or wortmannin in combination with TRAIL resulted in the decrease of BID (by 2.4- and 2.2-fold, respectively) although not effective with TRAIL alone (Fig. 4B). Similar results were obtained when J82/V, J82/G1, and J82/G3 were used (data not shown).
Thus, these results suggest that the PI3K/Akt pathway–related antiapoptotic block in galectin-3–overexpressing J82 cells occurs downstream of caspase-8, at the level of BID cleavage.

**Effects of phosphatidylinositol 3-kinase inhibition on Akt activity in galectin-3–overexpressing cells.** We examined the Akt activity in J82 transfectants. Akt activity was measured by Western blot analysis using an antibody that specifically recognizes the phosphorylated/activated form of Akt. Densitometric tracing analysis determined that the level of activated Akt in G1, G2, and G3 cells increased by 1.8-, 2.3-, and 1.9-fold, respectively. High expression of activated Akt was observed in J82/G1, J82/G2, and J82/G3 cells, whereas low expression of activated Akt was observed in parental J82 cells and J82/V cells. Total Akt levels among these clones were almost equal. We also measured the protein expression of TRAIL receptors (DR4 and DR5), and these protein expression levels were almost equal (Fig. 5A).

We next explored whether inhibition of PI3K by wortmannin and LY-294002 can inhibit constitutively active Akt. As shown in Fig. 5B, treatment of J82/G2 cells with wortmannin (1 μmol/L) or LY-294002 (25 μmol/L) for 24 hours reversed the high constitutive activity of Akt by 3.6- and 2.7-fold, respectively. Similar results were obtained when J82/G1 and J82/G3 cells were used (data not shown). The protein expression of DR4 or DR5 was not altered by treatment with these PI3K inhibitors.

These results strongly suggest that the PI3K inhibitor–induced TRAIL sensitization is predominantly dependent on the modulation of the Akt activity but not on the increase in the expression of TRAIL receptors in galectin-3–overexpressing J82 cells.

**Down-regulation of active Akt by phosphatidylinositol 3-kinase inhibitors renders galectin-3–overexpressing cells sensitive to tumor necrosis factor–related apoptosis-inducing ligand.** Finally, we examined the effects of down-regulation of active Akt on galectin-3–overexpressing cells by MTT assay. Cell viability assays showed that J82/G2 cells treated with wortmannin (1 μmol/L) or LY-294002 (25 μmol/L) in the presence of TRAIL (100 ng/mL) for 24 hours were very sensitive to TRAIL, to the same extent as parental J82 and J82/V cells (Fig. 5C). Similar results were obtained when J82/G1 and J82/G3 were used (data not shown).

The data revealed that down-regulation of Akt with PI3K inhibitors made galectin-3–overexpressing J82 cells sensitive to TRAIL killing.

Taken together, these results suggest that overexpression of galectin-3 confers TRAIL resistance to J82 cells by elevating Akt activity, and that Akt is an important regulator of TRAIL in these cells.

**Discussion**

Galectin-3 is a member of the galectin gene family that is expressed at elevated levels in a variety of neoplastic cell types and has been associated with cell growth, cellular adhesion process, cell proliferation, transformation, metastasis, and apoptosis (33–39). The expression of galectin-3 is up-regulated in various types of cancer. Several reports have indicated its involvement in carcinogenesis (40, 41). One possible reason for this is the antiapoptotic activity of galectin-3. Several reports have proposed mechanisms by which galectin-3 protects cells from apoptosis. Yang et al. (10) have reported that galectin-3 interacts with Bcl-2, and Takenaka et al. (27) have shown that nuclear export of galectin-3 is important for its antiapoptotic activity. Recently, we have shown that galectin-3 regulates the apoptotic pathway induced by CD95, a member of the TNF family (14). These facts prompted us to investigate the effect of galectin-3 on the apoptotic pathway induced by TRAIL, another member of the TNF family.
TRAIL is expressed in a number of tissues and displays potent apoptotic activity against selected targets including a variety of cancers (18, 42). In addition to its well-described effects on cell death, TRAIL can inhibit cell cycle progression whereas blockage of TRAIL results in hyperproliferation in autoreactive lymphocytes resistant to TRAIL-induced apoptosis (18). One of the kinases that mediate TRAIL-induced apoptotic pathway is Akt. Akt enhances cell proliferation and inhibits apoptosis in cancer cells. There is a variety of reports suggesting the role of Akt in chemotherapeutic resistance to apoptosis and indicating its prosurvival function (43). However, once expressed, active Akt is under tight regulation by PI3K and other kinases of the signaling pathway that promote cell survival (19, 20). Signaling of growth factors translocates Akt to the inner surface of the plasma membrane in proximity to regulatory kinases that phosphorylate and activate Akt (44). In this report, we have investigated the relationship between TRAIL sensitivity and the activation status of Akt in galectin-3–overexpressing J82 cells. We have found that the PI3K/Akt pathway is a key factor in TRAIL-induced apoptosis in these cells.
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Thus, it seems that constitutively active Akt in galectin-3–transfected J82 cells is an important regulator of TRAIL sensitivity, and that galectin-3 enhances PI3K/Akt activity, resulting in TRAIL resistance.

It has been documented in some reports that active Akt inhibits apoptosis by blocking BID cleavage, which is essential for cytochrome c release from the mitochondrial intermembrane space (44, 45). We found that galectin-3–transfected J82 cells did not show BID cleavage on TRAIL treatment alone, whereas TRAIL plus LY-294002 or TRAIL plus wortmannin induced BID cleavage.

Although ineffective alone, TRAIL in combination with LY-294002 or wortmannin induced a drop in ΔΨm and opened the permeability transition pore to release cytochrome c and subsequently activated caspase-9 in galectin-3–transfected J82 cells.

From the findings in this study, we propose the following model: TRAIL activated caspase-8 in both galectin-3–transfected J82 cells and control J82 cells, but only control J82 cells showed sensitivity to TRAIL. Caspase-3 can be directly activated by caspase-8 or mitochondrial events, which leads to apoptosis. Akt promotes cell survival by intervening in the apoptosis cascade upstream of cytochrome c release (BID cleavage level; ref. 44) and downstream of caspase-8 activation. Galectin-3 is mainly involved in the PI3K/Akt pathway, which mediates BID cleavage in this cascade (Fig. 6).

There is the evidence that galectin-3 contains the anti-death Asp-Trp-Gly-Arg (NWGR) motif, which is conserved in the Bcl-2 homology domain of the Bcl-2 family (7, 13), and this motif is shown to be critical for the antiapoptotic function of galectin-3 (13). The antiapoptotic effects of Bcl-2 and Bcl-XL have been extensively analyzed. Bcl-2 inhibits apoptosis against various toxic stresses through stabilization of ΔΨm (28, 29), blocking the release of apoptosis-inducer proteins such as cytochrome c from mitochondria to the cytoplasm, and thus inhibits the subsequent apoptosis-executing signaling events. It has been reported that Bcl-2 and Bcl-XL cannot inhibit TRAIL-induced apoptosis in leukemic cells (46, 47), suggesting tumor cells that have already acquired resistance to chemotherapeutic drugs by Bcl-2 and Bcl-XL can be killed by TRAIL, whereas Bcl-2 and Bcl-XL abrogated TRAIL-induced mitochondrial dysfunction and apoptosis in nonlymphoid cells (48, 49), suggesting a differential regulation of apoptosis by Bcl-2 and Bcl-XL. In this study, it is not clear whether Akt activated by galectin-3 has some effects on Bcl-2 to maintain mitochondrial homeostasis in the TRAIL-induced apoptosis cascade.

Previously, it was reported that galectin-3 overexpression in breast carcinoma cell line inactivated Akt by dephosphorylation and increased TRAIL-induced cytotoxicity (50). This result is the opposite effect of galectin-3 that we observed in this study. It is known that galectin-3 cannot function as an antiapoptotic factor in all cell types (51), so these inconsistencies should be due to use of different cell lines and different galectin-3–associated proteins. Recently, it has been reported that galectin-3 acts as a specific binding partner of activated K-Ras, and galectin-3 associated with activated K-Ras promotes strong activation not only of Raf-1 but also of PI3K (52). The expression of Akt at the protein level depends on the activation of such upstream kinases as PI3K (44), and taken together, these previous reports are consistent with our result. The PI3K signaling pathway has been implicated in the growth and apoptosis of various cell types (53, 54). We found that constitutively active Akt prevented the TRAIL induction observed with PI3K blockade, implicating the regulation of TRAIL expression by PI3K through Akt.

Figure 5. Effect of reconstitution of galectin-3 on Akt (Ser-473) phosphorylation and effect of PI3K inhibitors (LY-294002 and wortmannin) on cell viability of galectin-3–overexpressing J82 cells. A, cell lysates from J82 cells (P), vector control (V), and galectin-3–expressing cell clones (G1, G2, and G3) were subjected to immunoblotting with each antibody. B, immunoblot analysis of phospho-Akt levels in vector control (V) and galectin-3 transfectant (G2) cells incubated with PI3K inhibitors (wortmannin and LY-294002). The cells were treated with wortmannin (1 μmol/L) or LY-294002 (25 μmol/L) in the presence of TRAIL (100 ng/mL) for 24 hours. C, the J82/G2 cells were treated with (+) or without (−) wortmannin (W; 1 μmol/L) or LY-294002 (L; 25 μmol/L) in the presence of TRAIL (100 ng/mL) at various time points. Cell viability was examined by MTT assay. Points, mean; bars, SD. P, parental J82 cells; V, vector control J82 cells.

TRAIL (100 ng/mL) for 24 hours. C, incubated with PI3K inhibitors (wortmannin and LY-294002). The cells were further increased cell death of galectin-3–transfected J82 cells was observed only when TRAIL was combined with PI3K inhibitor LY-294002 or wortmannin.
Bladder cancer is among the most morbid of human malignancies. Thus, deeper understanding of the molecular mechanisms underlying bladder cancer progression will facilitate the development of more reliable drugs and treatment strategies that can be used to suppress the progression of the disease. In the urologic field, recent studies have reported that constitutively active Akt in prostate cancer cells may cause drug resistance, and Akt is the kinase which enhances cell proliferation and inhibits apoptosis in cancer cells. These are consistent with our findings and also prompt us to investigate the role of galectin-3 in the PI3K/Akt pathway in prostate cancer cells as a next aim.

In conclusion, we have shown a role of galectin-3 in the promotion and inhibition of TRAIL-induced apoptosis in human bladder cancer J82 cells. Galectin-3 promotes strong activation of the PI3K/Akt pathway in J82 cells, which confers TRAIL resistance to these cells. Constitutively active Akt is an important regulator of TRAIL in J82 cells, and cells having higher constitutively active Akt were more resistant to TRAIL-induced apoptosis. Our results may provide important insights into the understanding of galectin-3 and the apoptotic pathway induced by the TNF family of proteins.

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