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Glycogen Synthase Kinase-3 β : A Prognostic Marker and a Potential Therapeutic Target in Human Bladder Cancer

Sei Naito¹, Vladimir Bilim¹, Kaori Yuuki¹, Andrey Ugolkov³, Teiichi Motoyama², Akira Nagaoka¹, Tomoyuki Kato¹, and Yoshihiko Tomita¹

Abstract

Purpose: Although recent studies have shown glycogen synthase kinase-3 β (GSK-3 β), a serine/threonine kinase, as a positive regulator of pancreatic, colon, and kidney cancer cell survival and proliferation, the role of GSK-3 in bladder cancer remains unknown. Our objectives were to determine the subcellular localization of GSK-3 β and to evaluate the effect of GSK-3 inhibition in bladder cancer.

Experimental Design: We used immunohistochemical staining and nuclear/cytosolic fractionation to determine the expression pattern of GSK-3 β in human urothelial carcinomas. To study the effect of GSK-3 inhibition on bladder cancer cell proliferation and survival, we used pharmacologic inhibitors of GSK-3, RNA interference, MTS assay, bromodeoxyuridine incorporation assay, quantitative reverse transcriptase-PCR, and Western blotting.

Results: We found aberrant nuclear accumulation of GSK-3 β in 62% (43 of 69) and 91% (21 of 23) of noninvasive and invasive human urothelial carcinomas, respectively. GSK-3 β nuclear staining was significantly associated with high-grade tumors ($P < 0.001$), advanced stage of bladder cancer ($P < 0.05$), metastasis ($P < 0.05$), and worse cause-specific survival ($P < 0.05$) in bladder cancer patients. Moreover, we found that pharmacologic inhibition or genetic depletion of GSK-3 β resulted in decreased viability of bladder cancer cells.

Conclusions: Our results suggest nuclear accumulation of GSK-3 β as a novel prognostic marker in bladder cancer, show that GSK-3 contributes to urothelial cancer cell proliferation and survival, and identify GSK-3 as a potential therapeutic target in human bladder cancer. *Clin Cancer Res*; 16(21); 5124–32. ©2010 AACR.

An estimated 357,000 cases of bladder cancer were diagnosed in 2002 worldwide, making it the ninth most common cancer (1). There were 145,000 deaths, most of which occurred as the result of metastatic disease (1). Since the 1980s, methotrexate, vinblastine, doxorubicin, and cisplatin have been used as standard chemotherapy for metastatic urothelial cancer (2). Nevertheless, the median survival time of patients with metastatic disease is only about 13 months (2). Even if the metastatic lesions can be completely removed, the 5-year survival rate of

patients with metastatic bladder cancer is merely 33% (3). Identification of new therapeutic targets is warranted to develop better treatment approaches for urothelial carcinoma patients with metastasis.

Glycogen synthase kinase (GSK)-3 is a serine/threonine protein kinase that has two isoforms, GSK-3 α and GSK-3 β (4). GSK-3 was first described as a component of the glycogen synthase (GS) regulation through phosphorylation of GS (5). Based on the classic paradigm, GSK-3 β is a potential tumor suppressor as GSK-3 phosphorylates pro-oncogenic molecules such as c-Jun (6), c-Myc (7), CREB (8), NFATc (9), HSF-1 (10), cyclin D1 (11) and β -catenin (12), thereby targeting these molecules for a subsequent ubiquitin-proteasomal degradation. Recent reports have suggested, however, that GSK-3 positively regulates cancer cell proliferation and survival (13–23). Recent studies have shown that inhibition of GSK-3 activity suppresses viability of cancer cells in chronic lymphocytic leukemia (13), pancreatic cancer (14, 15), colon cancer (16, 17), ovarian cancer (18), thyroid cancer (19), glioblastoma (20), prostate cancer (21, 22), and renal cancer (23). However, the role of GSK-3 in human bladder cancer remains unknown.

In this study, we show aberrant nuclear accumulation of GSK-3 β in urothelial cancer cell lines and most bladder

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Translational Relevance

We found nuclear accumulation of glycogen synthase kinase (GSK)-3 β in most human bladder carcinomas, and this expression pattern was strongly associated with high-grade tumors, metastasis, and worse survival in bladder cancer patients. We show that inhibition of GSK-3 suppresses proliferation and survival of bladder cancer cells. Our results identify nuclear accumulation of GSK-3 β as a novel prognostic marker in bladder cancer, and suggest GSK-3 as a potential therapeutic target in urothelial carcinoma.

carcinomas. We found that GSK-3 β nuclear expression is associated with high-grade tumors, metastasis, and worse survival in bladder cancer patients. Moreover, we identify GSK-3 as a positive regulator of proliferation and survival of bladder cancer cells. Our results suggest GSK-3 β nuclear accumulation as a prognostic marker and identify GSK-3 as a potential therapeutic target in urothelial cancer.

Materials and Methods

Immunohistochemical staining

Surgical urothelial cancer specimens from 92 consecutive patients who underwent surgery (4 open radical cystectomies and 88 transurethral resections) between 2004 and 2007 at the Yamagata University Hospital were included in the study. The median age was 75.4 years. Patients without urothelial cancer for histologic type were excluded from this study. The study was approved by the Ethics Committee of Yamagata University, and all patients signed an informed consent form. The primary tumors were fixed in 10% buffered formalin and embedded in paraffin. Pathologic staging was determined according to the International Union against Cancer (UICC) tumor-node-metastasis (TNM) classification of malignant tumors. Pathologic grades were assigned according to a system developed by the Japanese Urological Association on the basis of UICC criteria in 1997.

Immunohistochemical staining was done as described (24). A monoclonal mouse antibody for GSK-3 β (clone 7; BD Transduction Laboratories) was used. Two 5- μ m-thick paraffin sections from two different parts of each tumor (representative of the entire tumor) were mounted on silanized glass slides (DakoCytomation Japan). After deparaffination and rehydration, epitopes were reactivated by autoclaving the sections in 10 mmol/L citric buffer (pH 6.0) for 10 minutes. The slides were incubated with the primary antibodies for 1 hour at room temperature in a moist chamber. After washing with PBS, the bound antibody was detected by the peroxidase method using Histofine simple stain MAX-PO (Nichirei). The staining reaction was developed by diaminobenzidine in the presence of H₂O₂. Nuclear counterstaining was done by hema-

toxylin. Positive and negative controls were included in each staining series. GSK-3 β nuclear expression was defined as staining of >10% of cell nuclei regardless of intensity of the cytoplasmic expression.

Cell culture and reagents

The established bladder cancer cell lines T24, SCaBER, HT1376, and RT4 were obtained from the American Type Culture Collection and were cultured as described previously (25). AR-A014418 (Sigma-Aldrich Japan) inhibits GSK-3 β in an ATP-competitive manner (*in vitro* IC₅₀ = 104 nmol/L) and does not significantly inhibit cyclin-dependent kinase (CDK) or other 26 kinases showing high specificity for GSK-3 β (26). SB-216763, an ATP-competitive GSK-3 inhibitor (Cayman Chemicals), inhibits GSK-3 *in vitro* with an IC₅₀ value of <100 nmol/L with no significant inhibition of 24 other protein kinases (27). TDZD-8, a non-ATP-competitive inhibitor of GSK-3 (IC₅₀ = 2 μ mol/L; Sigma-Aldrich Japan), does not inhibit protein kinases A or C, CK-2 or CDK1/cyclin B kinases at 4,100 nmol/L (28).

Western blotting analysis

Western blotting analysis was done as described previously (25). Fresh surgical specimens were snap frozen in liquid nitrogen and stored in deep freezer. The purity of tumor or normal tissues was confirmed by H&E staining of the sample. Normal counterpart was prepared from the normal portion of nephrectomized kidney from the same patient. For protein extraction frozen tissues were ground in liquid nitrogen and then processed as described previously (25). The horseradish peroxidase (HRP)-labeled second antibody was detected using the SuperSignal West Pico Substrate (Pierce) according to the manufacturer's instructions. We used β -actin as a loading control. The images were analyzed using the UN-SCAN-It gel Automated Digitizing System software (version 5.1 for Windows, Silk Scientific, Inc.). The following antibodies were used: Bcl-2 (DAKO, Japan); glycogen synthase and phospho-glycogen synthase from Cell Signaling Technology; GSK-3 β , poly(ADP-ribose) polymerase (PARP), and XIAP (BD Biosciences); GSK3 α (Upstate Cell Signaling Solutions); and β -actin (Abcam). Nuclear/cytosolic fractionation was carried out by the Dignam method as described previously (15).

RNA extraction and real-time reverse transcriptase-PCR

Total cellular RNA was extracted using the SV total RNA Isolation System (Promega) and first-strand DNA was synthesized using a cDNA Reverse Transcription Kit (Applied Biosystems Japan) following the manufacturer's instructions. Real-time quantitative reverse transcriptase-PCR (RT-PCR) was done in the 7300 Real Time PCR System (Applied Biosystems) using predesigned TaqMan Gene Expression Assays (Applied Biosystems) targeting human *Bcl-2* (Hs00236808_s1) mRNA and *XIAP* (Hs00236913_m1), and *GAPDH* (4352934E) was used as an endogenous control. Each experiment was repeated

at least three times to confirm reproducibility with the reaction in triplicate wells for each sample using a TaqMan Universal PCR Master Mix (Applied Biosystems) according to the standard protocol. The expression of the target mRNA was quantified relative to that of the *GAPDH* mRNA; untreated controls were used as a reference.

RNA interference

Transient knockdown of GSK-3 β was achieved in HT1376 and T24 cells using three siRNA: 42839, s6239, s6240 (its sequence - GGACAAGAGAUUUUAAGAAUtt, CUCAAGAACUGUCAAGUAAtt, CGAGAGCUCCAGAU-CAUGAtt; Applied Biosystems). Unrelated control siRNA was also used. Transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's recommendations.

Measurement of cell viability and cell proliferation

Cell viability was detected with a colorimetric assay, the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) using the tetrazolium compound according to the manufacturer's protocol. A bromodeoxyuridine (BrdUrd) cell proliferation assay (Calbiochem) was done according to the manufacturer's instructions.

Statistical analysis

Continuous variables are presented as means \pm SD. Continuous variables in the present study met the criteria for a normal distribution and were assumed to be parametric. They were analyzed using a two-tailed *t*-test or one-way ANOVA where appropriate. In case the groups were ordered and equally spaced, the posttest for a linear trend was used; otherwise the Tukey posttest was used to compare all pairs of values. Associations between immunohistochemical staining and pathologic or clinical characteristics were analyzed using Fisher's exact test for 2 \times 2 contingency tables or χ^2 test for larger tables. The χ^2 critical value for 0.05 probability level was 3.841. All χ^2 values exceeding 3.841 and *P* values <0.05 were considered to indicate statistical significance. Two-sided tests were used where applicable. Association between immunohistochemical staining and nonmetastatic rate/disease-specific survival were analyzed using the log-rank test with Kaplan-Meier curves. Analysis was done using DR.SPSS.

Results

Expression of GSK-3 β in bladder cancer cells

By Western blotting, we found GSK-3 β expressed in bladder cancer cell lines RT4, HT1376, T24, and SCaBER (Fig. 1A). We also detected the expression of phosphorylated glycogen synthase (pGS), a substrate of GSK-3, in these cell lines, indicating that GSK-3 is active in bladder cancer cells (Fig. 1A). Using nuclear/cytosolic fractionation, we found that GSK-3 β is expressed in the nucleus and cytoplasm of bladder cancer cells in RT4, HT1376, T24, and SCaBER cancer cell lines (Fig. 1B). We detected

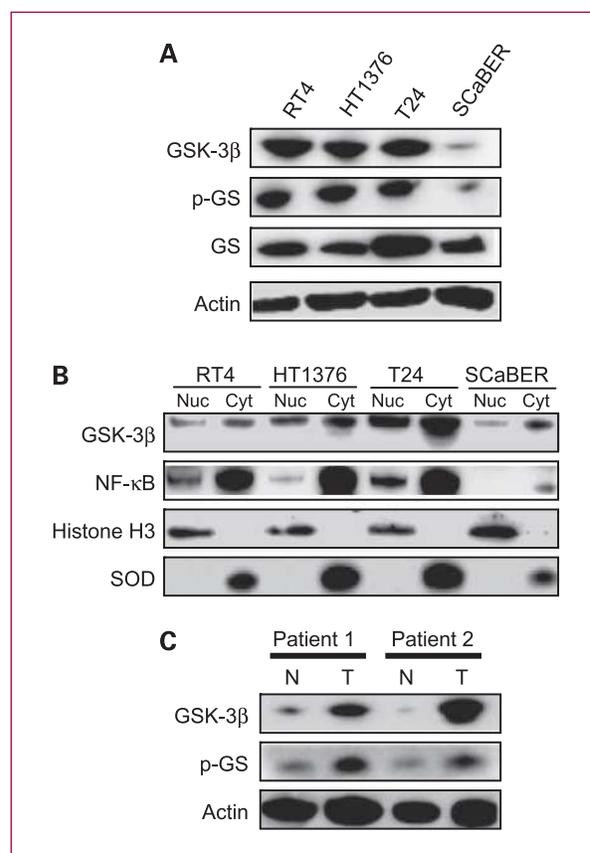


Fig. 1. GSK-3 β is expressed and active in human bladder cancer.

A, protein lysates from the indicated bladder cancer cell lines were separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membrane, and probed with antibodies against GSK-3 β , phospho-glycogen synthase (p-GS), and total glycogen synthase (GS). B, nuclear (Nuc) and cytosolic (Cyt) fractions were prepared from bladder cancer cell lines, separated by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies to the indicated proteins. Histone H3 and Cu/Zn superoxide dismutase (SOD) were used as nuclear and cytosolic markers, respectively. C, GSK-3 β and GS expression was detected in protein extracts from normal bladder (N) and primary urothelial carcinoma (T) obtained from bladder cancer patients.

nuclear expression of NF- κ B in RT4, HT1376, and T24 bladder cancer cell lines but not in SCaBER cancer cells that derived from squamous cell carcinoma (Fig. 1B). Using paired normal/tumor samples from bladder cancer patients, we found higher expression levels of GSK-3 β and pGS in tumors compared with their normal counterparts (Fig. 1C). Our results suggest high levels of GSK-3 β expression and activity in human bladder cancer.

GSK-3 β nuclear expression is associated with high-grade tumors, metastasis, and poor prognosis in human bladder cancer

Recently, we showed aberrant nuclear expression of GSK-3 β in pancreatic cancer, leukemia, and renal carcinomas (13, 15, 23). It has been shown that nuclear GSK-3 β might play a role in NF- κ B-mediated cancer cell survival (13, 15).

Although we found high levels and nuclear accumulation of GSK-3 β in bladder cancer cell lines, the expression pattern of GSK-3 β in cellular compartments in human bladder carcinomas is unknown.

Using immunohistochemical staining, we analyzed expression of GSK-3 β in 92 human urothelial cancer surgical specimens from bladder cancer patients (Table 1). We found weak cytoplasmic staining of GSK-3 β in benign bladder urothelial cells and in most low-grade tumors (Fig. 2A, Supplementary Fig. S1A-C). Nuclear accumulation of GSK-3 β was detected only in bladder cancer cells (Fig. 2B, Supplementary Fig. S1D-F). We found nuclear accumulation of GSK-3 β in 62% (43 of 69) of noninvasive tumors (<pT₂) and in 91% (21 of 23) of invasive bladder carcinomas (\geq pT₂ or N₊; Fig. 2C). We found GSK-3 β nuclear expression in grade 1 (G₁), grade 2 (G₂), and grade 3 (G₃) bladder carcinomas in 11 of 26 (42%), 18 of 28 (64%), and 35 of 38 (92%) cases, respectively (Fig. 2D). GSK-3 β nuclear staining was significantly associated with higher-grade tumors ($P < 0.001$, $\chi^2 = 18.61$; Fig. 2D) and advanced stage of bladder cancer ($P = 0.009$, $\chi^2 = 6.845$; Fig. 2C). GSK-3 β nuclear staining was detected in primary tumors from all three bladder cancer patients with lymph node metastasis. No patients died of cancer in the group in which the nuclear staining for GSK-3 β was negative. We found that GSK-3 β nuclear accumulation was significantly correlated with worse cause-specific survival in bladder cancer patients ($P = 0.043$; Fig. 2E). In addition, all patients with negative GSK-3 β nuclear staining did not have metastatic lesions within the follow-up period (median, 13.2 months) and showed no appearance of metastasis in the follow-up period, whereas 12 of 60 patients with positive GSK-3 β nuclear staining had *de novo* metastasis and this difference was statistically significant ($P = 0.029$; Fig. 2F). Our results show aberrant nuclear expression of GSK-3 β in most bladder carcinomas, and this expression pattern is associated with higher-grade tumors, metastasis, and worse survival in bladder cancer patients.

Table 1. Patient characteristics

No. of patients	92
Gender	
Male (%)	67 (72.8)
Female (%)	25 (27.2)
Median age (range), years	75.37 (41-94)
T	
T _{is}	4
T _a -T ₁	65
T ₂ -T ₄	23
Grade	
1	26
2	28
3	38
Median follow-up time (range), months	13.2 (1-43.7)

GSK-3 β positively regulates proliferation and survival of bladder cancer cells

Although we recently identified GSK-3 as a potential therapeutic target in pancreatic and renal cancer and in leukemia (13, 15, 23), the role of GSK-3 in bladder cancer is unknown. To test whether GSK-3 positively regulates proliferation and survival of bladder cancer cells, we used the small molecule inhibitor AR-A014418, which inhibits GSK-3 β kinase activity (IC₅₀ = 104 nmol/L). AR-A014418 does not significantly inhibit CDK2/CDK5 (IC₅₀ > 100 μ mol/L) and 26 other kinases, showing its specificity for GSK-3 β inhibition (26). We found that AR-A014418 suppresses the viability of bladder cancer cells in RT4, HT1376, T24, and SCaBER cell lines in a dose-dependent manner ($P < 0.0001$; Fig. 3A). Using BrdUrd incorporation assay, we found that pharmacologic inhibition of GSK-3 decreases cancer cell proliferation in RT4, HT1376, T24, and SCaBER urothelial cancer cell lines (Fig. 3B).

Moreover, we found that inhibition of GSK-3 increases the sub-G₁ cell population, an indicator of apoptosis, from 4% (control) to 31% (AR-A014418-treated cells) in T24 cancer cells (Fig. 3C). By Hoechst staining, we found that treatment with distinct small molecule inhibitors of GSK-3 β (AR-A014418, SB-415286, and TDZD-8) increases apoptosis in HT1376 cancer cell line (Supplementary Fig. S2). We had recently shown that GSK-3 β positively regulates NF- κ B-mediated expression of XIAP and Bcl-2 antiapoptotic molecules in pancreatic cancer, leukemia, and kidney tumors (13, 15, 23). Using nuclear/cytosolic fractionation, we found nuclear accumulation of NF- κ B, a marker of its activation, in RT4, HT1376, and T24 bladder cancer cell lines (Fig. 1B). Using real-time PCR, we investigated whether inhibition of GSK-3 affects expression of XIAP and Bcl-2 in bladder cancer cells. We detected that pharmacologic inhibition of GSK-3 led to a decreased expression of NF- κ B target genes *Bcl-2* and *XIAP* in HT1376 and T24 cancer cell lines (Fig. 4A). Consistent with these findings, we found that inhibition of GSK-3 downregulates XIAP and Bcl-2 protein expression in a dose-dependent manner in RT4, HT1376, and T24 cancer cells, leading to an increase in apoptosis as shown by PARP cleavage, a hallmark of apoptosis (Fig. 4B, Supplementary Fig. S3). To confirm that GSK-3 β positively regulates expression of antiapoptotic molecules and cancer cell viability in bladder cancer and this effect is specific to GSK-3 β , we used siRNA to deplete GSK-3 β expression in HT1376 cancer cells (Fig. 4C and D). We found that genetic depletion of GSK-3 β led to a decrease of XIAP and Bcl-2 protein expression in HT1376 cancer cells (Fig. 4C). Using MTS assay, we found that genetic depletion of GSK-3 β suppresses HT1376 bladder cancer cell viability (Fig. 4D).

These results suggest that GSK-3 positively regulates bladder cancer cell survival and proliferation.

Discussion

Recently, a number of studies showed GSK-3 as a potential therapeutic target in pancreatic, colon, renal, prostate,

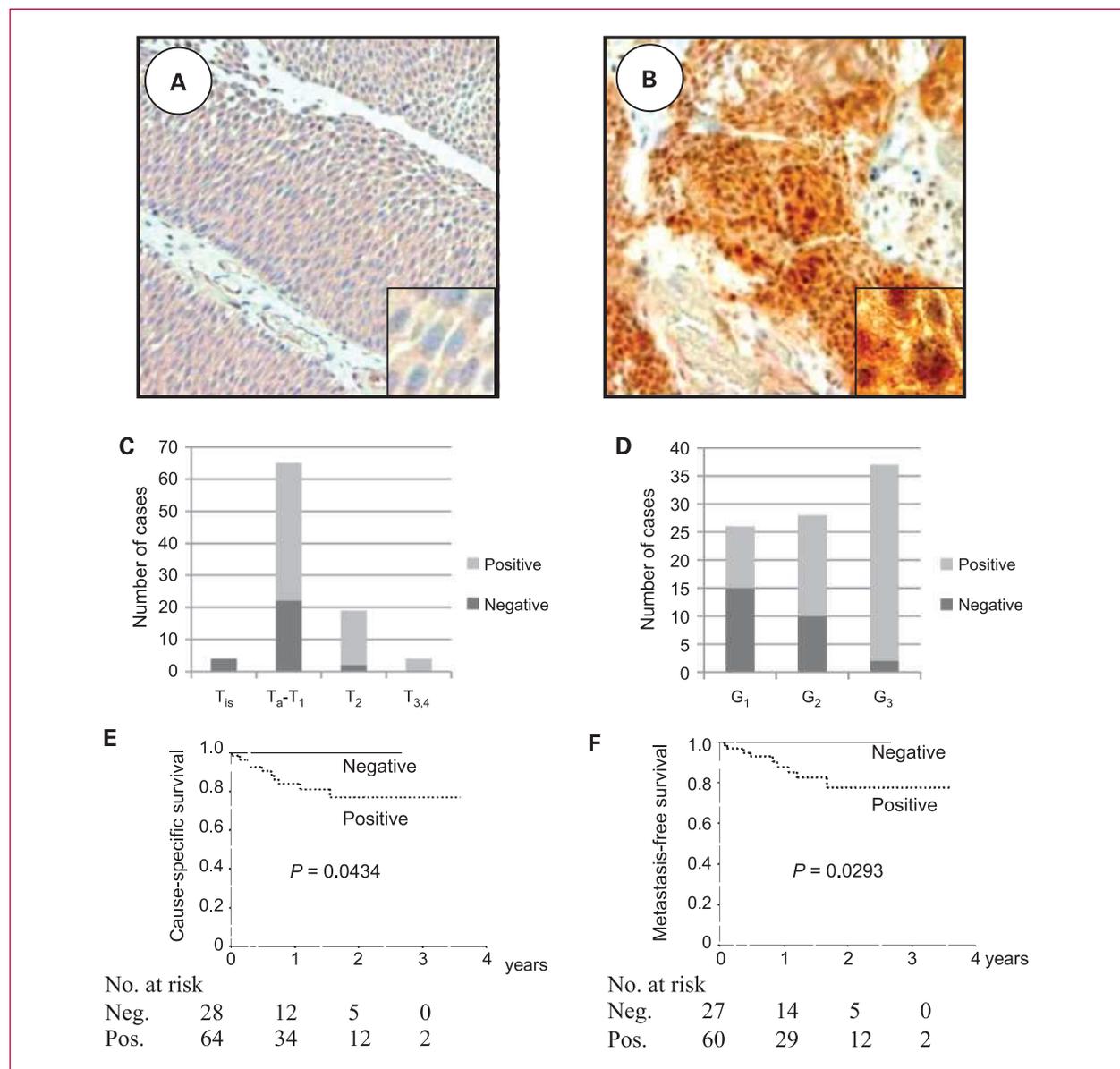


Fig. 2. GSK-3 β nuclear accumulation is associated with higher-grade tumors, metastasis, and worse survival in bladder cancer patients. A and B, immunohistochemical staining of GSK-3 β in low- (A) and high-grade (B) urothelial tumors. A, GSK-3 β cytoplasmic expression was detected in low-grade tumor. B, GSK-3 β nuclear staining was found in high-grade urothelial carcinoma. A and B insets, higher magnification view. C and D, distribution of GSK-3 β nuclear expression pattern in bladder carcinomas of different stage (C) and grade (D). E and F, vertical axes, survival rates. Kaplan-Meier survival curves show a statistically significant cause-specific (E) and metastasis-free (F) survival disadvantage in bladder carcinomas with GSK-3 β nuclear accumulation (indicated as positive).

brain, thyroid, and ovarian cancer and in leukemia (13–23). Nevertheless, the role of GSK-3 β in bladder cancer remains unknown. To the best of our knowledge, this is the first report on the role of GSK-3 β in urothelial cancer. Here, we show aberrant nuclear accumulation of GSK-3 β in urothelial cancer cell lines and most bladder carcinomas, whereas only weak cytoplasmic staining of GSK-3 β was detected in benign bladder tissues. Because nuclear expression of GSK-3 β was detected only in bladder cancer

cells, it might serve as a valuable marker in pathologic diagnosis of urothelial carcinoma. Previous studies support our findings of nuclear expression of GSK-3 β in bladder cancer cells (13, 15). It has been shown that GSK-3 β is accumulated in the nuclei of pancreatic and renal cancer cells, and malignant B cells (13, 15, 23). Similar to our findings in pancreatic cancer (15), we found that nuclear accumulation of GSK-3 β is strongly correlated with high-grade tumors in urothelial cancer. The prognosis

of bladder cancer varies, especially that of high-risk non-invasive bladder cancer (29). Early radical cystectomy for high-risk bladder cancer patients improves the prognosis, but compromises the quality of life (30). When preserva-

tion of the bladder is done in high-risk urothelial cancer patients, one third of the patients died of the disease (29). Identification of new biomarkers is warranted to predict a potential progression of bladder cancer and patient

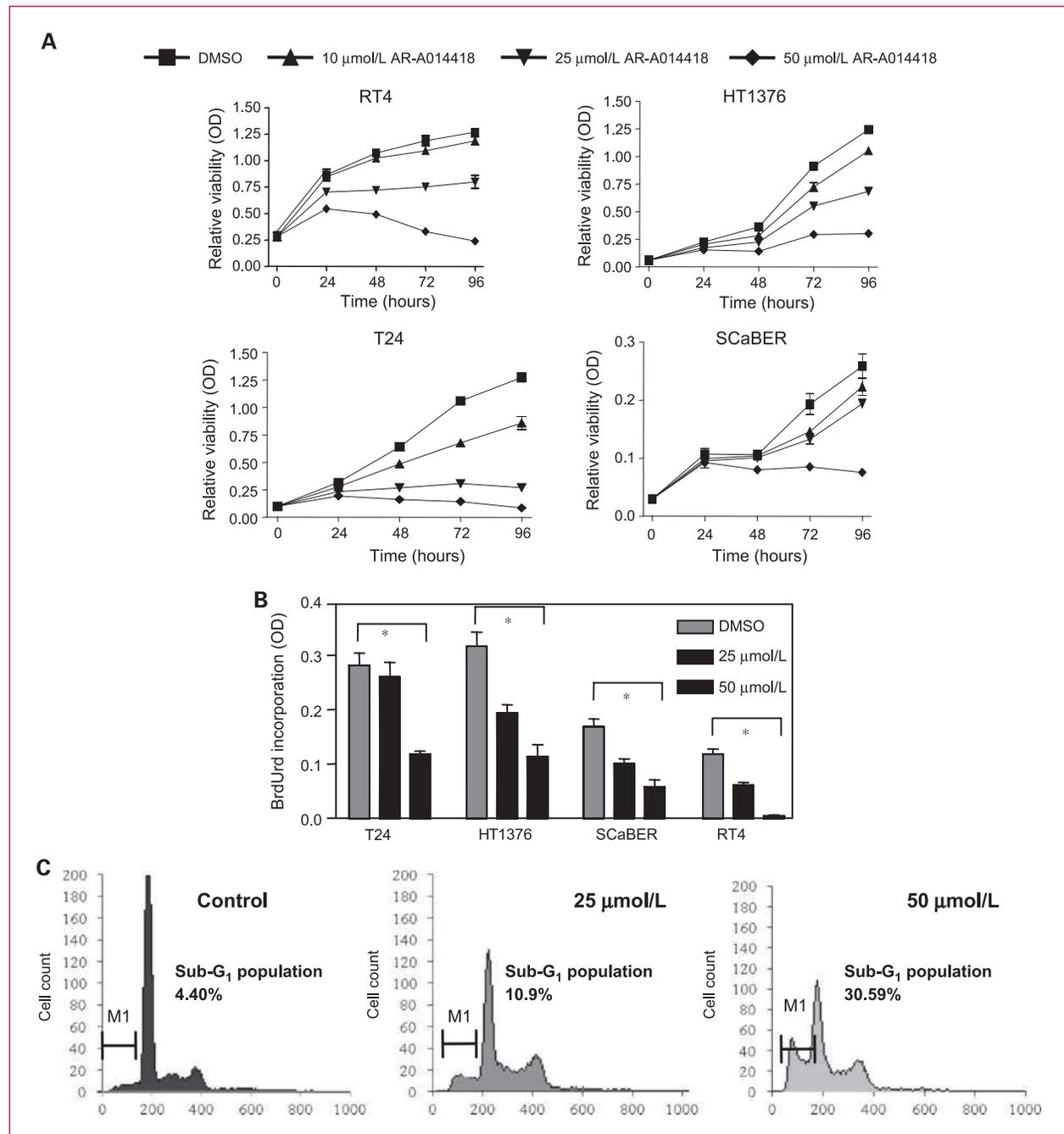


Fig. 3. Inhibition of GSK-3 suppresses viability of bladder cancer cells. A, relative cell viability was measured by MTS assay in T24, HT1376, RT4, and SCaBER urothelial cancer cell lines treated with indicated doses of AR-A014418 for 24, 46, 72 and 96 hours. OD, optical density. B, BrdUrd colorimetric assay was done after treatment of HT1376, RT4, and SCaBER urothelial cancer cells with AR-A014418 for 48 hours. The results are shown as OD 490 nm (ANOVA $P < 0.0001$; posttest for linear trend $P < 0.0001$). C, cell cycle distribution and appearance of sub-G₁ population are shown after treatment of T24 bladder cancer cells with indicated doses of AR-A014418 for 72 hours.

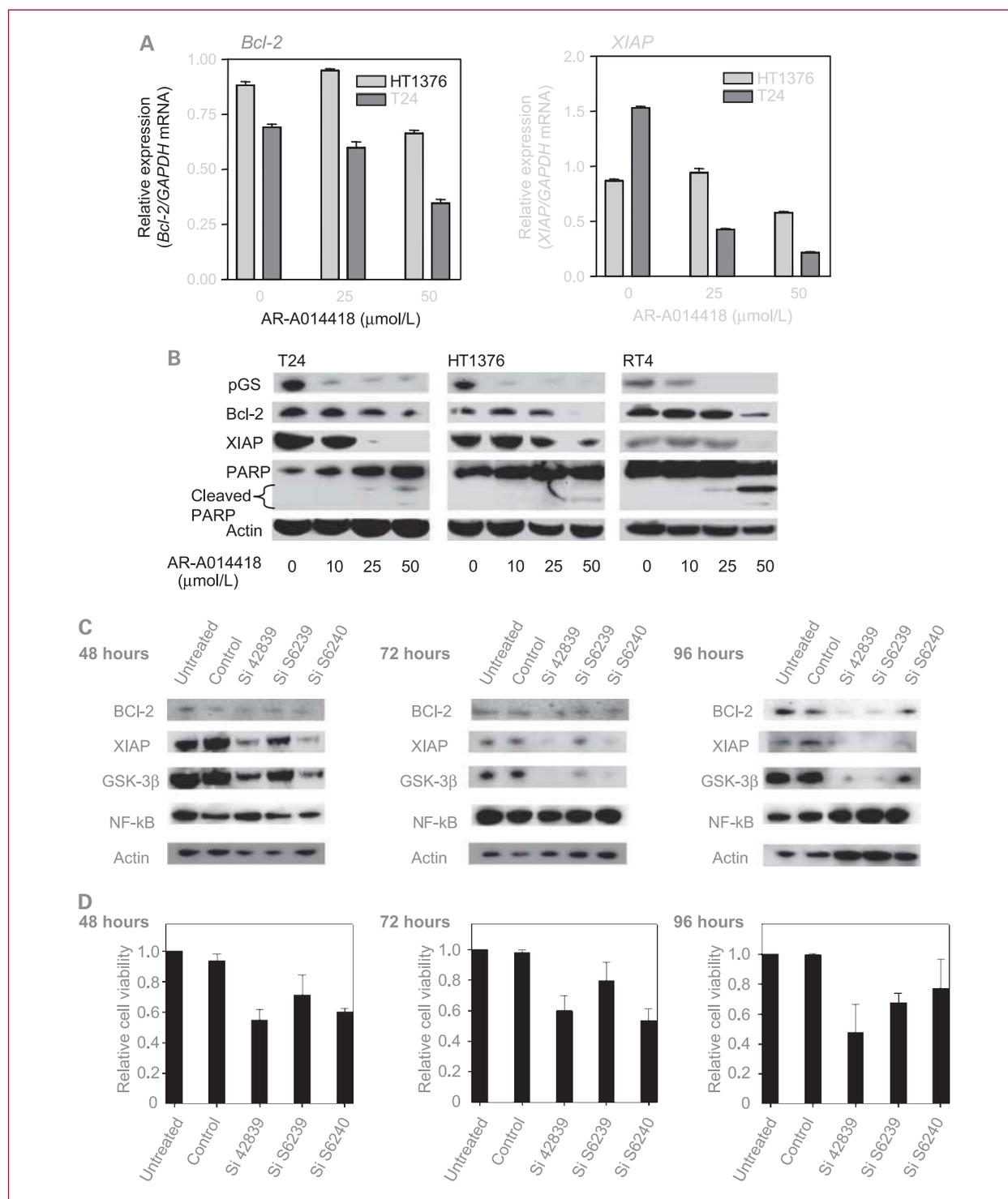


Fig. 4. Inhibition of GSK-3 decreases expression of XIAP and Bcl-2 in bladder cancer cells. A, HT1376 and T24 cancer cells were treated with indicated doses of AR-A014418; 24 hours after treatment, RNA was extracted from collected cells. Relative expression (normalized by GAPDH) of Bcl2 and XIAP is presented as measured by real-time PCR. B, T24, HT1376, and RT4 bladder cancer cells were treated with different concentrations of AR-A014418 as indicated; 72 hours after treatment, cell lysates were separated by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies to indicated proteins. C and D, HT1376 cancer cells were transfected with control siRNA and three distinct GSK-3 β siRNA constructs using Lipofectamine as described in the Materials and Methods; 48, 72 and 96 hours after transfection, cell lysates were separated by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies to indicated proteins (C), and relative cell viability was measured in transfected cancer cells by MTS assay (D); bars, SD.

survival. We found that GSK-3 β nuclear expression is associated with metastasis and worse survival in bladder cancer patients. Our study is the first to show a prognostic significance of GSK-3 β nuclear accumulation in human cancer. Our results suggest that GSK-3 β nuclear accumulation might serve as a useful prognostic marker in urothelial cancer.

Here, we identify GSK-3 as a positive regulator of proliferation and survival of bladder cancer cells. These findings are in agreement with previous studies showing that inhibition of GSK-3 leads to a decreased viability of pancreatic (15), leukemia (13), renal (23), colorectal (16), brain (31), and ovarian (18) cancer cells. Our results suggest GSK-3 as a potential new therapeutic target in human bladder cancer.

It has been reported that GSK-3 β is a positive regulator of NF- κ B transcriptional activity (13, 15). NF- κ B is known to play a role in human cancer progression and chemoresistance (32, 33), in part through positive regulation of its target genes *XIAP* (34) and *Bcl-2* (35). Levidou et al. showed that the nuclear expression of NF- κ B was correlated with the histologic grade and stage in bladder cancer (33). Our previous studies revealed that high expression of *Bcl-2* and/or *XIAP* defines urothelial carcinomas with higher metastatic potential and poor prognosis (25, 36). We have recently shown that inhibition of GSK-3 reduced *Bcl-2* and *XIAP* levels leading to a diminished survival of

leukemia and pancreatic and renal cancer cells (13, 15, 22). In agreement with our previous studies, we show that inhibition of GSK-3 downregulates *Bcl-2* and *XIAP* expression, leading to a decreased survival of bladder cancer cells. In this study, we found aberrant nuclear accumulation of GSK-3 β in urothelial cancer cell lines and most human bladder carcinomas. It has recently been shown that nuclear GSK-3 regulates histone modifications, and by this GSK-3 may contribute to NF- κ B p65/p50 binding to promoters and transcriptional activation of NF- κ B target genes *XIAP* and *Bcl-2* (15). Whether nuclear GSK-3 β affects histone modifications supporting NF- κ B transcriptional activity at promoters of *XIAP* and *Bcl-2* genes in bladder cancer cells remains to be investigated.

In summary, our work shows that GSK-3 β nuclear expression is associated with high-grade tumors, metastasis, and worse survival in bladder cancer patients. We identify GSK-3 as a positive regulator of proliferation and survival of bladder cancer cells. Our results suggest GSK-3 β nuclear accumulation as a prognostic marker and identify GSK-3 as a potential therapeutic target in human urothelial cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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