Expression of taxol resistance gene 1 correlates with gastric cancer patient clinical outcome and induces taxol resistance

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Abstract. In the present study, we investigated the effect of the taxol resistance gene 1 (TXR1) on taxol resistance in gastric cancer (GC). Immunohistochemistry was performed in order to assess the expression pattern of TXR1 in paraffin-embedded specimens of 107 GC patients who underwent radical D2 gastrectomy with long-term follow-up. In order to determine whether TXR1 expression plays a role in taxol resistance in GC cells, TXR1 was exogenously expressed or knocked down by siRNA in the absence and presence of taxol treatment, and cell proliferation was determined using the MTT assay. TXR1, thrombospondin-1, multidrug resistance protein mRNA and protein expression levels and certain drug resistance-related genes were determined by real time-PCR. There was a significant correlation between TXR1 expression and distant metastasis. Patients whose tissue biopsies tested negative for TXR1 expression had a higher post-operative 5-year survival rate than patients who had TXR1-positive tissue biopsies, even in the advanced cancer group. Exogenous expression of TXR1 in BGC823 cells induced taxol resistance, and siRNA knockdown of TXR1 sensitized human GC cells to taxol. The results show that low expression of TXR1 is correlated with a favorable prognosis in GC patients and that TXR1 likely plays a role in taxol resistance in GC cells.

Introduction

Gastric cancer (GC) is one of the most common malignant human tumors. Although the incidence of GC has recently decreased worldwide, it remains the second leading cause of cancer-related death and one of the most common malignancies in China, South America, Eastern Europe and Japan (1). GC patients are often at advanced stages when first diagnosed, and untreated advanced GC patients have a poor prognosis with a median survival time of 3-5 months (2). The current treatment regimen for advanced GC patients is surgery followed by chemotherapy and/or radiation therapy. After R2 radical treatment, 50-60% of locally advanced GC patients had recurrence and metastasis within 2 years. Adjuvant chemotherapy after surgery is important to further improve survival and reduce recurrence rates. In recent years, many pre-clinical trials for advanced GC patients have been performed with a variety of promising drug candidates, including docetaxel, taxol (paclitaxel), oxaliplatin, irinotecan and capecitabine, and these trials involved many taxol-based programs (3).

Taxol, a taxane, is a plant-derived alkaloid used as an anti-cancer chemotherapeutic. Taxol induces the assembly of tubulin into microtubules and then stabilizes the complex. This leads to cell cycle arrest at the metaphase/anaphase transition, due to the inability of the spindle and the spindle fibers to form during mitosis (4). As a single agent, taxol was found to induce tumor cell regression in 11-17% of patients who had no previous treatment (5); activity was also noted in previously treated patients (6). Earlier clinical Phase II trials revealed that taxanes are effective in the treatment of advanced GC and have no cross-resistance with other drugs and no additive or overlapping toxicity in the treatment of advanced GC. Therefore, many researchers are more likely to use taxanes combined with fluoropyrimidine and a cisplatin-based regimen to treat advanced GC. However, taxol resistance has limited its application (7).

Recently, Lih *et al* (8) identified the taxol resistance gene, taxol resistance gene 1 (TXR1), when screening for taxol resistance-associated genes in a prostate cancer cell line, M2182. TXR1, located at 12q12, encodes for a proline and serine-rich 15-kDa nuclear protein. TXR1 was found to be up-regulated 5-fold in taxol-resistant M2182 cells. Knockdown of TXR1 expression by siRNA restored sensitivity to taxol of these resistant M2182 cells. TXR1 was shown to induce taxol resistance by a mechanism independent of multidrug resistance protein (MDR1) and tubulin. TXR1 was shown to negatively regulate the transcript levels of secretion of apoptosis-inducing protein thrombospondin-1 (TSP1), which has been shown to induce taxol resistance. Papadaki *et al* examined the mRNA expression level of TXR1 and TSP1 in fresh tumor tissue from non-small cell lung cancer patients who underwent pre-operative chemotherapy. They found that the patients with high expression of TSP1 and low expression of TXR1 had a better clinical outcome (9). Therefore, targeting TXR1 may be beneficial in the treatment of GC patients.

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In the present study, we verified the relationship between TXR1 expression and the clinicopathological features and prognosis of GC patients, and explored the feasibility of TXR1 as a potential drug target in the treatment of this disease.

Materials and methods

Patients and follow-up. A group of 107 consecutive GC patients was studied. All patients were treated with radical D2 gastrectomy at the Peiking University People's Hospital and Beijing Friendship Hospital between January 2001 and June 2005. The study group consisted of 74 males and 33 females with a mean age of 59.46 years (range 19-87). All patients were tracked by direct evaluation or phone interview until death or until study end-date (June 2009), which provided a minimum of 4 years of follow-up. The study was approved by the Ethics Committee of Peking University People's Hospital and Beijing Friendship Hospital.

Tissue microarray and immunohistochemistry. Tissue arrays and immunohistochemistry were performed as described previously (10). In brief, slides for immunohistochemical analysis were deparaffinized, rehydrated and treated with 3% hydrogen peroxide solution. After antigen retrieval, the sections were incubated with primary antibodies overnight at 4˚C. Primary antibodies were detected using the Powervision two-step histostaining reagent (Zhongshan, Beijing), with PV-9001 as the secondary antibody; detection was performed by diaminobenzidine chromogenic reaction. Tissues were counterstained, dehydrated and mounted. Positive and negative controls were included. Two experienced pathologists independently examined, in a blinded manner, the protein stainings.

Constructed plasmids. TXR1-GFP was generated by subcloning the human TXR1 (NM_018457.2) cDNA clone (OriGene clone SC113472) by PCR into pEGFP-C3 (Clontech, Mountain View, CA, USA) using the following primers (5'-CCG *CTC GAG* ATG TGG AAT CCC AAT GCC -3' (forward) and 5'-CGC *GGA TCC* TCA GTC AGA ATC ACT GCT GGA-3' (reverse); 5'-*Xho*I and 3'-*Bam*HI sites are underlined. Clones were confirmed by sequencing.

Transfection. The human GC cell line, BGC823, was established at the Peking University People's Hospital and was cultured in complete DMEM medium (Hyclone, Omaha, NE, USA) containing 10% fetal calf serum (Gibco, Carlsbad, CA, USA) at 37˚C in a humidified atmosphere containing 5% CO_2. Cells were plated and grown to 70-90% confluency without antibiotics, and transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) as directed by the manufacturer. The cells were distributed into a 12-well plate, treated with G418 and after 2 weeks of selection G418-resistant cells were pooled for further analysis.

siRNA-mediated down-regulation of gene expression. Three sets of siRNA duplexes specific for TXR1 were chemically synthesized (Invitrogen): siRNA duplex 1, (sense) 5'-CAG UGA UAG UAG ACA AGA ATT-3, (anti-sense) 5'-UUC UUG UCU ACU AUC ACU GTT-3; siRNA duplex 2, (sense) 5'-GGU UAG AUC AUA UAG CUA ATT-3, (anti-sense) 5'-UUA GCU AUA UGA UCU AAC CTT-3'; siRNA duplex 3, (sense) 5'-GGA GGU UUG UGG AAA UUC ATT-3, (anti-sense) 5'-UGA AUU UCC ACA AAC CUC CTT-3'. A chemically synthesized mock siRNA (fluorescein-labeled, non-silencing) was also purchased from Invitrogen. Transfection of these oligos (50 nM) was performed using Lipofectamine 2000. For RNA extraction, cells were harvested 48 h after transfection. To measure drug cytotoxicity, cells were grown in 6-well plates and subsequently subcultured into 96-well plates 24 h after transfection.

Selection of taxol-resistance cell lines. The human GC cell line, BGC823, was maintained in DMEM containing 1% penicillin-streptomycin (Gibco) and 10% FBS. Selection for taxol-resistant BGC823 cells was performed (Concord Pharmaceutical, China) in a stepwise manner, essentially as previously described (11). BGC823 cells were initially exposed to 1 nM taxol and maintained at this concentration until cell growth returned to the same rate as the parental cell line, then the drug dose was increased. BGC823/T cells (taxol-resistant BGC823 cells) were maintained at a final concentration of 30 nM taxol.

Growth inhibition assays. Growth rates of cells in culture were determined using MTT assays (Sigma). Briefly, BGC823 cells were plated ($1x10^4$ cells/200 μl medium) in 96-well plates and grown under standard conditions for 24 h. Cells were treated with various concentrations of taxol, and 20 μl MTT (5 mg/ml) was added at the indicated time points after drug treatment. DMSO (100 μl; Sigma) was added to each well, and absorbance values were determined using a microplate reader (Bio-Rad) at 570 nm.

Quantitative real-time reverse transcription PCR. Total RNA from cells was prepared using TRIzol reagent according to the manufacturer's protocol (Invitrogen). cDNA libraries were generated by reverse transcription using M-MLV reverse transcriptase and Oligo dT primers (Promega, Madison, WI, USA). Quantitative PCR was performed using the Applied Biosystems Sequence Detection System 7900 and Power SYBR Green PCR MasterMix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Quantification of mRNA was performed using the ΔΔCT method. All PCR primers were designed online (http://frodo.wi.mit.edu and http://pga.mgh.harvard.edu/primerbank) (Table I).

Western blot analysis. Total protein was extracted from cells in the logarithmic phase of growth and quantified using the BCA method (Pierce). Equal amounts of protein were resolved by 12-15% SDS-PAGE and electro-transferred to PVDF membranes using Mini Protean 3 systems (Bio-Rad). PVDF membranes were blocked with PBS containing 5% fat-free milk powder for 2 h, then incubated with primary antibodies [TXR1 rabbit anti-human polyclonal antibody (a gift from Professor S. Cohen, Stanford University) (8) and mouse anti-human β-actin monoclonal antibody (Sigma)] overnight at 4˚C. Membranes were washed with PBS and subsequently incubated with anti-mouse or anti-rabbit IgG secondary antibodies conjugated to horseradish peroxidase, and peroxidase activity was visualized using enhanced chemiluminescence.

Table I. Primer sequences for real-time PCR analysis.

Gene	Primer	Sequence (5'→3')	Product (bp)
TXR1	Forward	GGACCCTTCCCTCAAGTCTC	157
	Reverse	CTCTTCCCATTTCCCCTAGC	
TSP1	Forward	GCTGGTGGTAGACTAGGGTTGTTT	143
	Reverse	CCAGAAGGTGCAATACCAGCAT	
BCRP	Forward	TGAGCCTTTGGTTAAGACCG	107
	Reverse	TGGTGTTTCCTTGTGACACTG	
GSTP1	Forward	ACCTCCGCTGCAAATACATC	98
	Reverse	CTCAAAAGGCTTCAGTTGCC	
MDR1	Forward	GGCTCCGATACATGGTTTTCC	76
	Reverse	CCAGTGGTGTTTTTAGGGTCATC	
MRP1	Forward	GTTTCTCAGATCGCTCACCC	102
	Reverse	TCCACCAGAAGGTGATCCTC	
MVP	Forward	CTGGGAGTTGGTGGTGATCT	379
	Reverse	CAACTGGCACTTTGAGGTGA	
TOP2A	Forward	TTTGACCACGCGGAGAAG	170
	Reverse	GAGTCCATCAGATTTGTGGAA	
GAPDH	Forward	CCTGCCAAGTATGATGACATCAAGA	66
	Reverse	GTAGCCCAGGATGCCCTTTAGT	

Statistical analysis. All data were analyzed using SPSS11.0 software. The numeration data were analyzed by a paired-sample t-test or independent sample t-test. The association of protein expression with various clinicopathological features was analyzed using the Chi-square test. Cumulative survival was estimated by the Kaplan-Meier method, and differences between survival curves were analyzed using a log-rank test. P-values <0.05 were considered statistically significant.

Results

Expression of TXR1 correlates with the clinicopathological features of GC patients. The group of 107 patients with GC ranged in age from 19 to 87 years (mean 59.46±12.95) and was comprised of 74 males and 33 females. Follow-up was carried out for at least 5 years. The most favorable prognoses were noted for patients with intestinal type (P=0.011), well-differentiated (P=0.014), pTNM stages I and II (P=0.002) GC, without lymph node metastasis (P=0.032) and without distant metastasis (P=0.014), compared to patients with other types of GC cancers (Table II).

Immunohistochemical analysis showed that TXR1 was found in the nucleus and cytoplasm of GC cells in 71% (76 of 107) of the paraffin-embedded biopsies. We observed that TXR1 expression was present in normal gastric mucosa and in intestinal metaplasia samples (Fig. 1). In the normal gastric mucosa, TXR1 was expressed only in the cell nucleus, while in dysplasia, TXR1 expression was located in the nucleus and cytoplasm of the cells.

There was significant correlation between TXR1 expression and distant metastasis (no metastasis vs. distant metastasis, P=0.040). The expression of TXR1 was not found to correlate with age, tumor location, Lauren classification, differentiation, pTNM stage, depth of wall invasion or lymph node metastasis (Table II).

Expression of TXR1 correlates with the clinical outcome of GC patients. GC patients with tissue biopsies with TXR1-negative cells had a higher post-operative 5-year survival rate (67.7%) than those with tissue biopsies with TXR1-positive cells (42.1%, P=0.091; Fig. 2A). Cases were divided into early GC and advanced GC groups, and the expression of TXR1 was analyzed and compared to clinical outcome. In advanced GC, the 5-year survival rate of TXR1-negative patients was significantly higher than that of TXR1-positive patients (64.3 vs. 33.3%, P=0.007; Fig. 2B). Cox regression analysis of the 91 patient samples demonstrated that TXR1 expression (P=0.007), TNM stage (P=0.01) and Lauren classification (P=0.026) were independent prognostic indicators (Table III).

Overexpression of TXR1 increases resistance to taxol. To elucidate the contribution of TXR1 to taxol resistance, BGC823 cells were transfected with pEGFP-TXR1 and selected with G418 (400 μg/ml) for 14 days. Expression of exogenous TXR1 was validated by Western blot analysis (Fig. 3A). Sensitivity to taxol (10 and 100 nM) was examined in the BGC283 cells using MTT assays. The expression of TXR1 did not effect cell growth in the absence of treatment, as both untransfected and transfected BGC823 cells exhibited the same growth rates (Fig. 3D). There were also no obvious differences in morphology between the two cell lines.

TXR1, TSP1, BCRP, GSTP1, MDR1, MRP1, MVP and TOP2A mRNA expression levels were determined in the BGC823 and BGC823-TXR1 cells (Fig. 3B). The exogenous

Table II. Clinicopathological features and nuclear TXR1 expression in the tissue of GC patients.

	No. of cases	Prognosis			TXR1		
		Survival	Death	P-value	Negative	Positive	P-value
Gender				0.326			0.041
Female	33	14	19		14	19	
Male	74	39	35		17	57	
Age (mean, 59.46±12.95 years)				0.040			0.217
≤55	42	26	16		15	27	
≥56	65	27	38		16	49	
Location				0.238			0.325
Corpus and fundus	34	14	20		12	22	
Antrum	73	39	34		19	54	
Lauren classification				0.011			0.113
Intestinal type	89	49	40		23	66	
Mixed or diffuse type	18	4	14		8	10	
Differentiation				0.014			0.424
Poor or undifferentiated	65	26	39		17	48	
Moderate or well	42	27	15		14	28	
pTNM stage				0.002			0.427
I	16	14	2		3	13	
II	20	11	9		6	14	
III	47	22	25		17	30	
IV	24	6	18		5	19	
Depth of wall invasion				0.006			0.461
T1	11	9	2		2	9	
T2	11	8	3		2	9	
T3	67	32	35		23	44	
T4	18	4	14		4	14	
Lymph node metastasis				0.032			0.697
No	34	22	12		9	25	
Yes	73	31	42		22	51	
Distant metastasis				0.014			0.040
No	92	50	42		30	62	
Yes	15	3	12		1	14	

expression of TXR1 was associated with an increase in TXR1 mRNA, a decrease in TSP1 mRNA and an increase in the drug resistance BCRP, GSTP1, MDR1, MVP and TOPO II genes.

BGC823-TXR1 cells were approximately 40-fold more resistant to taxol compared to the BGC823 cells (Fig. 3C, E and F). Treatment with different concentrations of taxol and for different periods of time showed that the growth of BGC823-TXR1 cells was uninhibited by treatment. Therefore, TXR1 expression in BGC823 cells enhanced resistance to taxol.

Knockdown of TXR1 increases taxol sensitivity of human GC cells. To determine whether the anti-cancer activity of taxol is enhanced by inhibition of TXR1, three TXR1 siRNAs (siRNA1, 2 and 3) were transiently transfected into BGC823/T cells, and TXR1 levels were evaluated 48 h later. To minimize non-specific effects, 50 nM of each siRNA was used, and each siRNA was separately transfected into the BGC823/T cells. As a result, an 80% decrease in TXR1 expression was observed following transfection of siRNA1 or siRNA2 (Fig. 4A). Transfection with the negative control had no effect on TXR1 expression. Notably, TSP1 mRNA levels were increased in the BGC823 cells transfected with TXR1 siRNAs, while MDR1 mRNA levels decreased (Fig. 4A). To evaluate the taxol sensitivity of these siRNA-treated cells, the survival rate following incubation with taxol was compared to untreated control cells. The BGC823/T cells were approximately 40-fold more resistant to taxol compared to the BGC823 cells, and BGC823/T-siRNA cells were approximately 10-fold more sensitive to taxol than the BGC823/T cells (Fig. 4B). A significant enhancement in the chemosensitivity of the BGC823/T cells transfected with siRNA was observed with siRNA1 and siRNA2 transfection ($P<0.01$), and the effect was greater than with siRNA3 transfection ($P<0.01$, Fig. 4C). Given that siRNA1 and siRNA2 transfection resulted in a greater decrease in TXR1 expression than siRNA3 transfection,

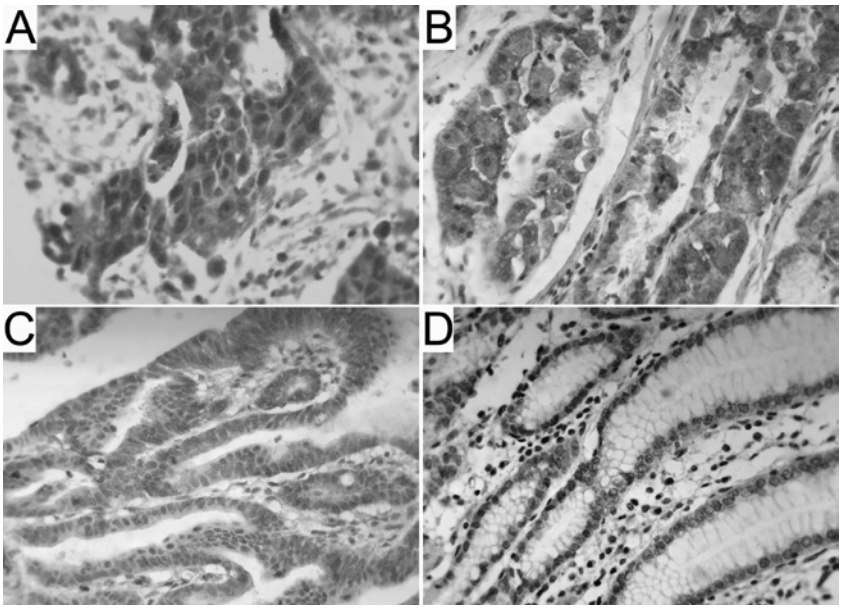

Figure 1. Expression of TXR1 in gastric tissue. Immunohistochemical staining on tissue was performed (brown chromogenic reaction): poor differentiated gastric cancer (A); well-differentiated gastric cancer (B); dysplasia (C); normal gastric mucosa (D). In the normal gastric mucosa, TXR1 was expressed only in the cell nucleus, while in cancer and dysplastic tissues TXR1 expression was located in the cell nucleus and cytoplasm. Original magnification x400.

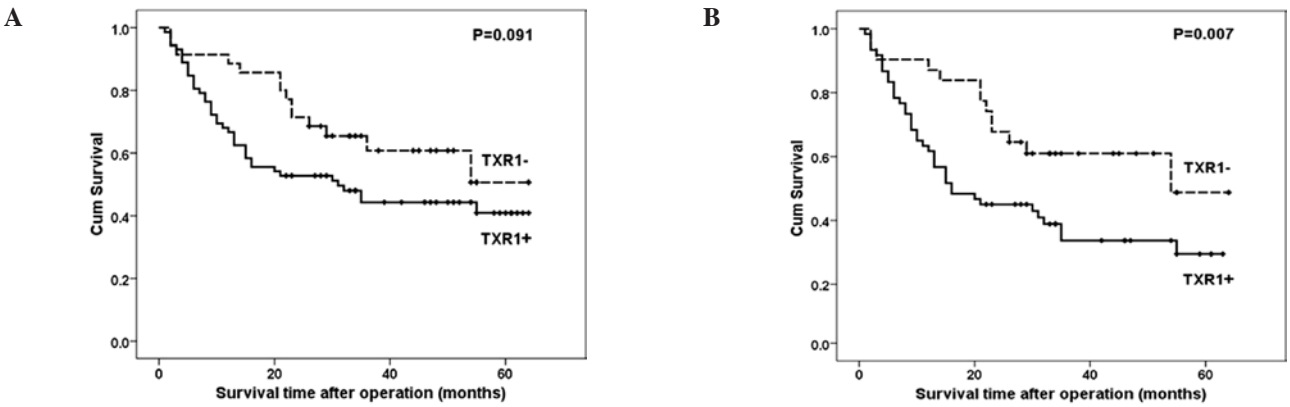

Figure 2. Kaplan-Meier survival curves for the 107 gastric cancer patients receiving radical D2 gastrectomy and categorized by TXR1 expression. (A) All patients, P=0.091. (B) Advanced GC patients, P=0.007. Survival was significantly more favorable for advanced GC patients with negative TXR1 expression (TXR1-) than for TXR1-positive (TXR1+) patients.

Table III. Multivariate analysis of the prognostic factors of the GC patients using the Cox proportional hazard model.

	B	SE	Wald	df	P-value	Exp (B)	95% CI for Exp (B)	
							Lower	Upper
Lauren classification								
Diffuse vs. intestinal type	0.763	0.344	4.936	1	0.026	2.146	1.094	4.208
TNM stage			11.304	3	0.010			
II vs. I	1.536	0.786	3.819	1	0.051	4.647	0.995	21.691
III vs. I	1.937	0.741	6.833	1	0.009	6.938	1.624	29.651
IV vs. I	2.367	0.766	9.553	1	0.002	10.667	2.378	47.856
TXR1								
Positive vs. negative	0.991	0.366	7.316	1	0.007	2.694	1.314	5.524

B, β regression coefficient; SE, standard error; Wald, test statistics used for the determination of the meaning of variables; df, degrees of freedom; Exp (B), exponent; CI, confidence interval. Variables included in the logistic regression model were gender, age, location, Lauren classification, differentiation, TNM stage, invasion depth, lymph node metastasis, distant metastasis and expression of TXR1.

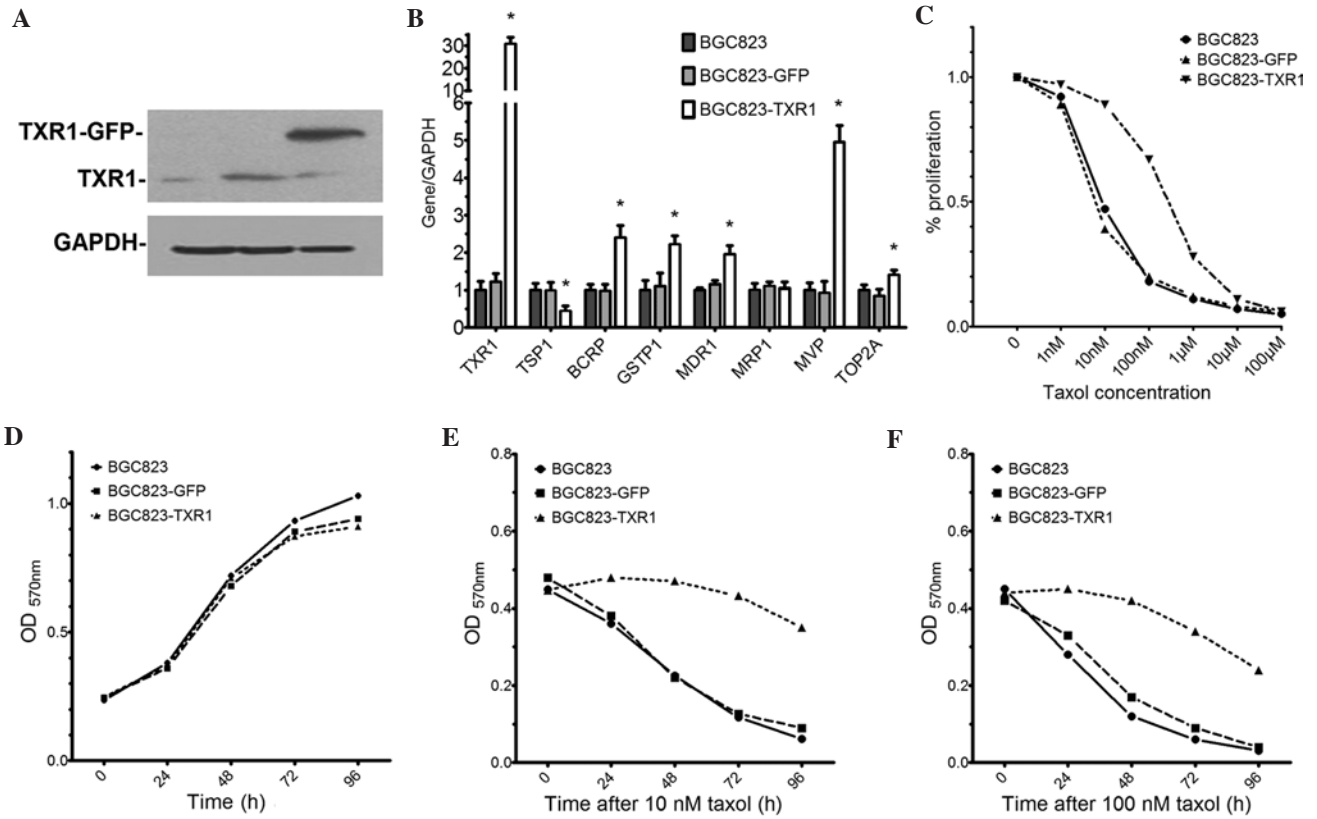

Figure 3. Exogenous expression of TXR1 in BGC823 cells increases resistance to taxol. (A) Expression of TXR1 protein after transfection with a TXR1 expression vector or a corresponding empty vector. (B) mRNA levels of TXR1, TSP1, BCRP, GSTP1, MDR1, MRP1, MVP and TOP2A were detected using real-time PCR and normalized to GAPDH for the BGC823, BGC823-GFP and BGC823-TXR1 cell lines. (C) MTT assays were used to monitor the proliferation of the BGC823, BGC823-GFP and BGC823-TXR1 cell lines in the presence of taxol. (D) Growth rates for untransfected and transfected BGC823 cells as determined by MTT assay. (E and F) MTT assays for BGC823, BGC823-GFP and BGC823-TXR1 cell lines treated with 10 and 100 nM taxol.

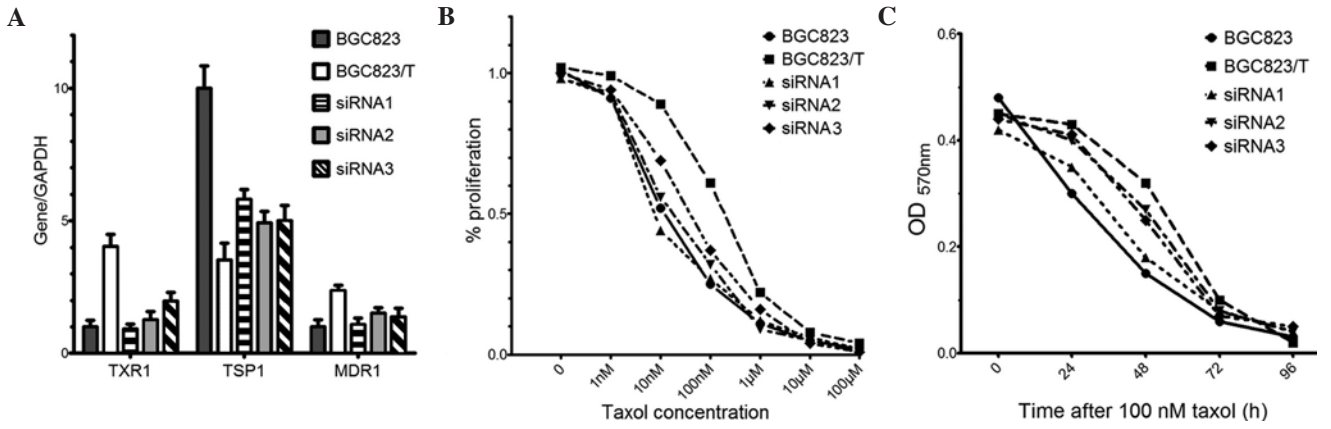

Figure 4. Sensitization of GC cells to taxol by inhibition of TXR1. (A) TXR1, TSP1 and MDR1 mRNA levels were determined by real-time PCR and normalized to GAPDH in BGC823, BGC823/T cells and BGC823/T cells transfected with TXR1-targeted siRNAs. (B) MTT assays were performed to monitor the proliferation of BGC823, BGC823/T and BGC823/T-siRNA cell lines treated with the indicated concentrations of taxol for 24 h. (C) MTT assays were used to evaluate the growth effect of 100 nM taxol treatment on the proliferation of BGC823, BGC823/T and BGC823/T-siRNA cell lines over time.

these data provide further evidence that the expression level of TXR1 is associated with taxol resistance.

Discussion

In the present study, we found that inhibition of TXR1 sensitized GC cells to taxol, a taxane. Increased expression levels of TXR1 in tumor tissue were shown to be correlated with poor prognosis and distant metastasis in GC patients. Furthermore, exogenous expression of TXR1 increased taxol resistance in BGC823 cells. Inhibition of TXR1 expression by siRNA restored taxol sensitivity to resistant cells.

Tissue microarray technology is a high-throughput tool that enables a rapid and concurrent analysis of molecular

targets in a large number of specimens at the DNA, RNA and protein levels under standardized conditions (12). Using this technology, combined with immunohistochemical analysis, we analyzed 107 pairs of tissue samples from GC patients. We found that decreased expression levels of TXR1 in GC patients correlated with favorable prognosis and the absence of distant metastasis. Similarly, Papadaki *et al* examined lung adenocarcinoma samples from patients treated with a docetaxel/gemcitabine combination and found that patients with low TXR1 and high TSP1 expression had higher survival rates compared to patients with high TXR1 and low TSP1 expression (9).

Although the expression of a variety of known resistance-associated molecules, such as MDR1, MRP, GST-π, P-gp and Topo II, has been defined in GC (13), Fan *et al* found that, in drug-resistant GC patients, the expression rates of some of these known resistance molecules was not increased (14). Therefore, it appears as though drug resistance in GC patients is very complex and may involve multiple signaling networks. To date, there is no effective clinical method for reversing multidrug resistance in patients; therefore, drug resistance mechanism(s) and new multi-drug resistance-associated molecules need to be identified in GC patients.

Cases of taxol resistance emerged soon after its application in the clinic, and the mechanism of resistance appears to be complex as several pathways have already been shown to contribute to this process (15). First, the tubulin isoform gene mutation is one of the major determinants of taxol resistance in patients with non-small cell lung cancer (16). It has been demonstrated that changes in microtubule protein, tubulin isoforms and polymerization kinetics may be related to taxol resistance (17). Another mechanism of taxol resistance involves the P-gp, increased drug efflux pump (18). Modulation of the activity of proteins involved in apoptosis, including bcl-2, p53, erbB2, PKC and telomerase, may also be related to taxol resistance (19). Therefore, mechanisms of taxol resistance require further study, as the present study has not clearly defined this process (20).

TXR1 is an important complement to the mechanism of taxol resistance. Lih *et al* (8) first characterized the *txr1* gene as encoding a nuclear protein that played a role in taxol resistance. They also demonstrated that TXR1 inhibits TSP1 expression at the transcriptional level, thereby, preventing taxol-induced apoptosis in human prostate cancer cells. Inactivation of TXR1 by CD47 expression and activation of TSP1 were also shown to enhance taxol-induced cytotoxicity in human prostate cancer cells. TXR1 induction of taxol-resistance was found to be independent of MDR1 and tubulin. A high expression level of TXR1 did not alter the intracellular accumulation of [3H]-labeled taxol in a taxol-resistant human prostate cancer cell line. In our study, exogenous expression of TXR1 was associated with an increase in both MDR1 and TSP1.

To understand the effect of TXR1 on the drug resistance of GC cell lines, we increased and decreased TXR1 expression to delineate its role. We observed that exogenous expression of TXR1 induced taxol resistance in the GC cell line, BGC823, while decreasing TXR1 expression restored the taxol sensitivity of these cells. Therefore, we considered that TXR1 expression may contribute to taxol resistance. We found that when TXR1 was exogenously expressed, resistance to taxol treatment increased and the expression levels of BCRP, GSTP1, MDR1, MVP and TOPO II increased. Furthermore, transient transfection of TXR1-targeted siRNAs was also shown to restore the chemosensitivity of MCF-7 cells (unpublished data).

Tissue microarray technology is a powerful tool for efficiently scanning a range of potential tumor biomarkers. Combining tissue microarray and immunohistochemical analysis, we found that increased expression of TXR1 correlated with certain clinicopathological characteristics of GC patients, as well as clinical outcome. This area warrants further study to determine how the function of TXR1 may contribute to clinicopathological features, such as distant metastasis and poor clinical outcome. Additionally, the consequences of TXR1-induced TSP1 require further investigation and validattion in GC patients.

Moreover, we found that TXR1 protein was located in the nucleus in normal gastric cells and in the nucleus and/or cytoplasm in tumor cells. The significance of TXR1 expression in the cytoplasm of tumor tissue and the relationship between TXR1 and tumorigenesis are significant issues which require further study.

In summary, the present study shows that low expression levels of TXR1 are correlated with favorable prognosis in GC patients; TXR1 was a significant factor for taxol resistance in the GC cell line, BGC823, and may be a potential target for the treatment of taxol resistance.

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