Thioredoxin-interacting protein: an oxidative stress-related gene is upregulated by glucose in human prostate carcinoma cells

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Abstract

Thioredoxin-interacting protein (TXNIP), also known as vitamin-D₃ upregulated protein 1, interacts with reduced thioredoxin. This protein modulates the cellular redox state and plays a role in stress-induced cellular apoptosis. This study examined *TXNIP* gene expression in prostate cancer cells. *In vitro* studies by immunoblot assay have shown that elevated glucose levels (1–15 mM) upregulate *TXNIP* gene expression two- to fourfold in human prostate carcinoma cells (LNCaP) and hepatocellular carcinoma cells (HepG2). Transient gene expression assays reveal that the promoter activity of the *TXNIP* gene is upregulated by glucose, 3-*O*-methylglucose, and maltose, but not by mannitol. These results suggest that glucose and 3-*O*-methylglucose induce TXNIP expression through both glucose metabolism-dependent and -independent pathways. Cotransfection of a plasmid expression carbohydrate response element-binding protein (ChREBP) with a TXNIP reporter vector into LNCaP cells dramatically enhances reporter activity in a low glucose (1 mM) condition. The effects of glucose are apparently mediated in a region located –341 to –324 bp upstream of the translational starting point of the *TXNIP* gene as indicated by 5'-deletion and site-directed mutagenesis reporter assays. Mutation of the putative carbohydrate response element (ChoRE) from CACGAGGGCAGCAGAG to TTTGAGGGCAGCAGAG abolishes glucose upregulation of TXNIP promoter activity. The present study demonstrates that TXNIP is transcription induced in both LNCaP and HepG2 cells in an increased glucose metabolism-dependent or -independent response, and a putative glucose regulatory system including ChREBP and ChoRE is needed for glucose-induced *TXNIP* gene in human prostate carcinoma cells.

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Introduction

Thioredoxin-interacting protein (TXNIP), also known as vitamin-D₃ upregulated protein 1 (VDUP1) or thioredoxin-binding protein-2 was first isolated from HL-60 cells following 1,25-dihydroxyvitamin D₃ treatment (Chen & DeLuca 1994). TXNIP is known to negatively regulate thioredoxin (TRX) that further regulates mammalian signal transduction (Gulati *et al.* 2001, Nishiyama *et al.* 2001). Therefore, recent studies have gradually clarified the involvement of TXNIP in important cellular events such as vascular inflammation and proliferation (Schulze *et al.* 2002, Yamawaki *et al.* 2005), collagen expression in mesangial cells (Kobayashi *et al.* 2003), melanoma metastasis (Goldberg *et al.* 2003), mucosal immunity in gastrointestinal cancer (Ikarashi *et al.* 2002), osteoclast differentiation (Aitken *et al.* 2004), cardiac hypertrophy, and myocardial ischemia (Wang

et al. 2002, Yoshioka *et al.* 2004, Xiang *et al.* 2005), decidualization of endometrium (Simmons & Kennedy 2004), renal carcinogenesis (Dutta *et al.* 2005), T-cell leukemia leukemogenesis and immunology (Nishinaka *et al.* 2004, Lee *et al.* 2005), lung growth (Filby *et al.* 2006), cell proliferation, and the aging process (Yoshida *et al.* 2005, Wang *et al.* 2006). Since TXNIP expression is often suppressed in tumor cells, this protein apparently has tumor-suppressive properties. TXNIP is a novel factor in p27^{kip1} stability via regulation of JAB1. Transfection of TXNIP induces cell cycle arrest at the G0/G1 phase in several types of tumor cells (Han *et al.* 2003, Jeon *et al.* 2005). However, TXNIP antisense-transfected murine melanoma cells exhibit decreased levels of intracellular reactive oxygen species and inhibited cell proliferation (Song *et al.* 2003). TXNIP function and gene regulation in human prostate cells require further clarification. Early studies have indicated

that TXNIP expression is negatively regulated by androgens in murine prostate cells (Pang *et al.* 2002).

Recent studies have indicated that TXNIP is important in metabolic responses to feeding. Oligonucleotide microarray analysis from two independent laboratories indicated that TXNIP is a glucose-upregulating gene in human pancreatic islets and rat-1 fibroblasts (Hirota *et al.* 2002, Shalev *et al.* 2002). Analyses of HcB-19 mice or TXNIP knockout mice have also revealed the critical role of TXNIP in the integrated regulation of glucose and lipid metabolism during fasting (Hui *et al.* 2004, Sheth *et al.* 2005, Oka *et al.* 2006). Furthermore, a recent study of genome-wide expression profiles has indicated that the human TXNIP gene is important in regulating peripheral glucose metabolism (Parikh *et al.* 2007). A study using human pancreatic β -cells further revealed glucose stimulation of TXNIP expression. Transient gene expression indirectly suggests that glucose induces TXNIP expression through a putative carbohydrate response element (ChoRE) that does not contain the consensus sequence of ChoRE in the promoter of the TXNIP gene (Minn *et al.* 2005). Interestingly, this study indicated that upregulation of the TXNIP gene by glucose does not require glucose metabolism.

This study characterizes TXNIP expression in human prostate carcinoma cells and the regulatory mechanism of glucose on TXNIP expression.

Materials and methods

Cell cultures and chemicals

Prostate cancer cell lines PZ-HPV-7, CA-HPV-10, LNCaP, PC-3, DU145, and hepatocarcinoma cells (HepG2) were obtained from the American type culture collection and maintained as described previously (Feng *et al.* 2005, Tsui *et al.* 2008). PZ-HPV-7 cells were derived from epithelial cells cultured from normal tissue from the peripheral zone of the prostate. CA-HPV-10 cells were derived from prostatic adenocarcinoma cells of Gleason grade 4/4. The cells were immortalized by transformed human papillomavirus (Weijerman *et al.* 1994). Glucose, maltose, mannitol, and 3-O-methylglucose were purchased from Sigma. FCS was purchased from HyClone (Logan, UT, USA). All culture media and reagents were purchased from Life Technologies. Cells were cultured in RPMI 1640 containing 10% FCS, and the medium was replaced twice a week. In the following experimental studies, cells were cultured in RPMI1640 glucose-free medium with 10% FCS for 4–6 h to glucose starvation and then cultured in the same medium with various concentrations of glucose for 16–24 h.

Semi-quantitative reverse transcription-PCR

Total RNA was isolated with Trizol reagent; cDNA was synthesized by the superscript III preamplification system, following the manufacturer’s instructions (Life-Technologies). Excess RNA was degraded by RNase H treatment. TXNIP-P and TXNIP-R primers (Table 1) were used to amplify sequences specific to human TXNIP mRNA (GenBank accession no. NM_012391). The quality of cDNA was verified by controlled reactions using primers derived from β -actin-P and β -actin-R (Table 1). PCR was performed in a thermal cycler (Thermolyne, Dubuzue, IA, USA) under the following parameters: 30 cycles at 94 °C for 0.5 min, 55 °C for 1 min and 72 °C for 1 min. PCR products, 615 bp of TXNIP cDNA fragments and 720 bp of β -actin cDNA fragments, were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

Expression vector constructs

The c-Myc (MGC:5184) and ChoRE-binding protein (ChREBP; MGC:9556) cDNA vectors were purchased from the Human Genome Mapping Project Resource Centre, UK. Human c-Myc cDNA was linearized by cutting with *Kpn I* and *Xho I* and ligation with the overexpression vector pcDNA3 (Invitrogen), resulting in insertion of c-Myc cDNA in the polyadenylate region controlled by the CMV promoter (pcDNA3-c-Myc). Purchased ChREBP cDNA (MGC:9556) vector does not contain full-length ChREBP cDNA but it does have 279 bp deletion (exon 5 and exon 6). Therefore, the full-length human ChREBP overexpression vector was constructed by digested ChREBP cDNA vector with *Eco RV* plus *Xmn I* and *Bas HI* plus *Xho I*, which led to two cDNA fragments, 344 and 2645 bp. Another 553 bp cDNA fragment was synthesized using RT-PCR from HepG2 cells by two primers ChREBP-P and ChREBP-R (Table 1) and digested with *Xmn I* and *Bas HI*. These three cDNA fragments were ligated and cloned into the pcDNA3 overexpression vector after digestion with *Eco RV* and *Xho I*. Proper ligation was confirmed by extensive restriction mapping and sequencing.

Table 1 Primers used in this study for RT-PCR

Name	Sequence
TXNIP-P	5'-AGTTTCCTGCATGTTTCATTCCT-3'
TXNIP-R	5'-GAAGTTGAACTCAGGGGCATAC-3'
β -actin-P	5'-GAAGATCAAGATCATTGCTCCTCC-3'
β -actin-R	5'-GAAGATCAAGATCATTGCTCCTCC-3'
ChREBP-P	5'-CCAAGTGAAGAATTTCAAAGG-3'
ChREBP-R	5'-CGGGAGTTGGTAAAGAAATCTG-3'

Immunoblot assay of human *TXNIP* and β -actin

Cells were treated with different glucose concentrations as indicated. Cells were lysed with lysing buffer (62.5 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 5% β -mercaptoethanol and 7 M urea), and protein concentrations of aliquot samples were measured by bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL, USA). Equal amounts of protein were loaded onto a 12.5% SDS-polyacrylamide gel and assayed by ECL detection per manufacturer instructions (Amersham Biosciences). The blot membrane was probed with 1:1000 diluted monoclonal *TXNIP* antiserum (JY2; MBL International Corp., Woburn, MA, USA) or 1:1000 diluted anti β -actin antiserum (C11, Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Reporter vector constructs and site-directed mutagenesis

The reporter vector of the *TXNIP* gene (−3104 to −1) was provided by Dr I. Choi, Korea Research Institute of Bioscience and Biotechnology, Republic of Korea (Han *et al.* 2003). The reporter vector containing the 5'-flanking region of the human *TXNIP* gene was cloned by PCR using the GL-2 primer and *TXNIP*-specific primers (Table 2). Reporter vectors, pChoREm1, and pChoREm2, containing the mutant putative ChoRE were established using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). The double-stranded primers used for *in vitro* site directed mutagenesis were 5'-GGAGCACACCGTCCCCACGCGCCACAGCGATC-3' and 5'-CAATGGGAGGATGTGTTTGGGGCAGCACGAGCC-3' respectively (mutation sites are underlined).

Transient transfections and reporter assay

The LNCaP or HepG2 cells were plated onto 24-well plates at 1×10^4 cells/well one day prior to transfection.

Table 2 Primers used in this study for construction of reporter vectors

Name	Sequence	Site
TXNIP-2	5'-ACGCGTTACACTTTCATACC-CATC-3'	−1614
TXNIP-3	5'-ACGCGTACTCAGCTCCTAAAG-CATC-3'	−1102
TXNIP-4	5'-ACGCGTAGAAATTGAAGAGAT-GACAGG-3'	−921
TXNIP-5	5'-ACGCGTCCA-CACCCGCGCTCCGATG-3'	−612
TXNIP-5-1	5'-ACGCGTCAA-CAGCCCCCTCCTTCCCC-3'	−524
TXNIP-5-2	5'-ACGCGTGTCCACGCGCCA-CAGCGA-3'	−420

Cells were transiently transfected using the TransFast transfection reagent (0.6 μ g/well; Promega Biosciences) with 1 μ g/well of reporter vector and 0.5 μ g/well of the pCMVSPORT β Gal (Life Technologies) for 4 h. Cells were then incubated in RPMI 1640 medium with 10% FCS overnight. Transfected cells were treated with RPMI 1640 glucose-free medium with 10% FCS for 4 h, and then with the same medium at varying concentrations of glucose, as indicated, for an additional 16 h. Cell extracts were assayed for luciferase and β -galactosidase (β -GAL) activity according to the manufacturer's instructions (Promega Biosciences). Luciferase activity was determined by LumiCount (Packard Bioscience, Meriden, CT, USA) and adjusted accordingly for β -GAL enzymatic activity, as previously described (Juang *et al.* 2004). However, preliminary studies indicated that cotransfection with high concentrations of ChREBP or c-Myc affects the activity of the β -galactosidase expression vector (pCMVSPORT β gal; Life Technologies) in transient gene expression assay. Therefore, luciferase activity in these experiments was adjusted using protein concentrations in the whole cell extract, which were measured by bicinchoninic acid protein assay (Pierce Biotechnology).

Electrophoretic mobility shift assay

An electrophoretic mobility-shift assay (EMSA) was performed as described previously (Tsui *et al.* 2006). The double-stranded DNA fragment containing the putative ChoRE (5'-TGTGCACGAGGGCAGCAGAGCCTC-3'), was 5'-end labeled with γ -P³²ATP using T4 polynucleotide kinase. Nuclear extracts of LNCaP cells, after treatment with different glucose concentrations, were obtained using NE-PER nuclear and cytoplasmic extraction reagents, per manufacturer instructions (Pierce, Rockford, IL, USA). The 5'-end-labeled ChoRE (ChoRE probe; 5 nM) was incubated with 2 μ g nuclear extract (NE) from LNCaP cells in 20 μ l binding buffer (25 mM HEPES buffer (pH 7.9), 50 mM KCL, 0.05 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 10% glycerol) containing 0.5 μ g poly (dI-dC)-poly(dI-dC). The binding shift was challenged with 50-fold double-stranded ChoRE or double-stranded mutational ChoREm (5'-TGTGTTT-GAGGGCAGCAGAGCC-3'), without labeling the 5'-end with γ -P³²ATP. Protein–DNA complex formations were analyzed on 4% polyacrylamide gels by autoradiography.

Statistical analysis

Results were expressed as the mean $\pm$ S.E.M. for at least three independent replications of each experiment. Statistical significance was determined by paired *t*-test analysis and one-way ANOVA using SigmaStat software for Windows (version 2.03; SPSS Inc., Chicago, IL, USA).

Figure 1 Gene expression of *TXNIP* in various prostate carcinoma cells. The *TXNIP* gene expression in different cell lines was determined by (A) RT-PCR and (B) immunoblot assay. PZ, PZ-HPV-7; CA, CA-HPV-10; LN, LNCaP; PC, PC-3; and DU, DU145. Quantitative analysis was done by determining the intensity of each band for *TXNIP* and β -actin from three independent experiments. Band intensity was analyzed by the GeneTools program of ChemiGenius (Syngene, Cambridge, UK). Data are presented as the mean density of *TXNIP*/ β -actin ($\pm$ S.E.M.) relative to the mean density associated with the PZ-HPV-7 cells.

Results

We screened *TXNIP* expression in five prostate cell lines (PZ-HPV-7, CA-HPV-10, LNCaP, PC-3, and DU145). To synchronize the cell cycle, all prostate cells used in this study were incubated in RPMI media without serum for 24 h and incubated with RPMI media containing 10% FCS for another 24 h. RT-PCR (Fig. 1A) and immunoblot assays (Fig. 1B) revealed a high rate of metastasized prostate carcinoma cells (PC-3 and DU145), thus indicating a lower amount of *TXNIP* gene expression, when compared with non-metastatic (PZ-HPV-7 and CA-HPV-10) or low-metastatic prostate cells (LNCaP). Transient gene expression and immunoblot assays revealed that elevated glucose (1–15 mM) induced a two- to fourfold upregulation in *TXNIP* gene expression in human prostate carcinoma cells, LNCaP (Fig. 2A and D). When a plasmid expression ChREBP was cotransfected with the *TXNIP* reporter vector into LNCaP cells, promoter activity was dramatically ($P < 0.001$) enhanced in a low glucose (1 mM) condition. However, overexpression of c-Myc did not significantly ($P = 0.076$) induce *TXNIP* promoter activity in the low glucose (1 mM) condition (Fig. 2B and C). Glucose-upregulated *TXNIP* gene expression was observed not only in prostate cells but also in the HepG2 cells (Fig. 3A and D). Promoter activity was significantly ($P < 0.001$) enhanced when a plasmid expression ChREBP was cotransfected with a *TXNIP* reporter vector into HepG2 cells (Fig. 3B). However,

transient overexpression of c-Myc did not significantly ($P = 0.382$) induce *TXNIP* promoter activity in the low glucose (1 mM) condition (Fig. 3C).

The effects of glucose are apparently mediated via a region located -420 to -1 bp upstream of the translational starting point of the *TXNIP* gene according to 5'-deletion reporter assays (Fig. 4A). Two putative ChoRE sequences, -430 to -414 GAGCA-CACCGTGTCCAC and -341 to -324 CACGAGGG-CAGCAGCAG (putative E-boxes underlined), were observed within this region. Two mutant reporter vectors, pChoREm1 and pChoREm2, were used to determine the specific ChoRE on the *TXNIP* gene. Following mutation of the putative -341 to -324 ChoRE from CACGAGGGCAGCAGCAG to TTT GAGGGCAGCAGCAG transient gene expression assays revealed that the reporter vector pChoREm2, but not pChoREm1, with the ChoRE mutation blocked the effect of 15 mM glucose treatment (Fig. 4B and C). These results indicate that the effects of glucose are mediated by a region located -341 to -324 bp upstream of the translational starting point of the *TXNIP* gene. Transient gene expression assays revealed that the promoter activity of *TXNIP* gene was upregulated by treatment with glucose or maltose but not with mannitol. The promoter activity of *TXNIP* was upregulated fourfold when LNCaP cells were treated with high concentrations (30 mM) of 3-O-methylglucose, although 3-O-methylglucose does not undergo phosphorylation by glucokinase (Fig. 5A). These results

Figure 2 Effects of glucose on human *TXNIP* gene promoter activity in LNCaP cells. Reporter vector containing 5'-flanking region (−1614 to +1) of *TXNIP* gene was constructed and transient gene reporter assay was performed as described in 'Materials and methods'. Experimental data are presented as mean ($\pm$ S.E.M.; $n=6$) (A) luciferase activity of reporter vectors. Regulation of *TXNIP* gene promoter activity by different glucose concentrations as indicated in LNCaP cells. Promoter activity after transient (B) overexpression of ChREBP or (C) c-Myc in LNCaP cells. (D) Cells were treated with different concentrations of glucose (as indicated) for 24 h, then harvested and lysed to extract protein for immunoblot assay. (*: $P<0.05$; **: $P<0.01$).

were also found in HepG2 cells. Both concentrations (15 and 30 mM) of 3-*O*-methylglucose treatment significantly induced *TXNIP* promoter activity in comparison with the 1 mM 3-*O*-methylglucose-treated group (Fig. 5B). Results of immunoblot assays also revealed similar findings (Fig. 5C). The effects of 3-*O*-methylglucose may be mediated via the same putative ChoRE with glucose treatment since mutation of the ChoRE from GAGCACACCGTGTCCAC (−430 to −414) to ACACCGTCCCCACGCG did not abolish the activating effects of 30 mM 3-*O*-methylglucose on *TXNIP* promoter activity. However, mutation of the ChoRE from CACGAGGGCAGCACGAG (−341 to −324) to TTTGAGGGCAGCACGAG blocked 50% of the activating effects of 30 mM 3-*O*-methylglucose on *TXNIP* promoter activity in LNCaP cells (Fig. 5D). Similar results were also found using the HepG2 cells (data not shown). The glucose-upregulated promoter activity of the *TXNIP* gene was not abolished when cells were cotreated with 10 mM of the cell-permeable antioxidant (*N*-acetylcysteine) and glucose (Fig. 5E). These findings suggest that *TXNIP* is transcription-induced by increased metabolism-dependent or -independent responses, but not by increased oxidants.

Electrophoretic mobility shift assays indicated the specific binding characteristics of the ChoRE in the

promoter region of the human *TXNIP* gene. The nuclear extract from LNCaP cells treated with varying glucose concentration (1 and 15 mM) specifically bonded to the P³² labeled double-stranded oligonucleotide containing the putative ChoRE in different intensities. The observed mobility shift intensity was decreased significantly when the reaction mixture was challenged with unlabeled double-stranded oligonucleotides containing the ChoRE (Fig. 6).

Discussion

TXNIP mediates oxidative stress via suppression of TRX, a thio reductase with many cellular functions (Junn *et al.* 2000, Nishiyama *et al.* 2001). *TXNIP* is involved in several biological cellular events, especially cellular viability, through interaction with TRX (Schulze *et al.* 2002, Wang *et al.* 2002, Yoshida *et al.* 2005). A clinical study has indicated that glucose intolerance is associated with the high TRX levels in the plasma of diabetes mellitus patients (Miyamoto *et al.* 2005). Moreover, several *in vivo* and *in vitro* studies have revealed that deregulated TRX causes abnormal nutrition signals, which indicates that *TXNIP* is involved in the integrated regulation of glucose and

Figure 3 Effects of glucose on human *TXNIP* gene promoter activity in HepG2 cells. Reporter vector containing 5'-flanking region (–1614 to +1) of *TXNIP* gene was constructed and transient gene reporter assay was performed as described in Fig. 2. (A) Regulation of *TXNIP* gene promoter activity by different concentrations of glucose as indicated in HepG2 cells. Promoter activity after transient (B) overexpression of ChREBP or (C) c-Myc in HepG2 cells. (D) Cells were treated with different concentrations of glucose (as indicated) for 24 h. Cells were harvested and lysed to extract protein for immunoblot assay. (*: $P < 0.05$; **: $P < 0.01$).

Figure 4 Glucose treatment modulation of human *TXNIP* gene is dependent on the putative carbohydrate response element. (A) 5'-deletion assay shows that glucose regulation of *TXNIP* gene promoter activity depends on the (–524 to +1) DNA fragment. (B) The *TXNIP* promoter-luciferase reporter pTXNIP (–524 to +1) vector or mutant *TXNIP* promoter-luciferase reporter pChoREm1 or (C) pChoREm2 was transfected into LNCaP cells and transfected cells were treated with 1 mM glucose (black bars) or 15 mM glucose (white bars) for 16 h. Experimental data are presented as mean percentage ($\pm$ S.E.M.; $n = 6$) luciferase activity produced by 15 mM glucose treatment relative to 1 mM glucose-treated samples. (**: $P < 0.01$).

Figure 5 Modulation by glucose, 3-*O*-methylglucose, and antioxidants of TXNIP promoter activity. TXNIP (–524 to +1) reporter vector-transfected (A) LNCaP cells and (B) HepG2 cells were treated with different concentrations (white boxes, 1 mM; gray boxes, 15 mM; black boxes, 30 mM) of glucose, maltose, 3-*O*-methylglucose (3-*O*-mGLU), and mannitol for 24 h. (C) LNCaP cells (left) and HepG2 cells (right) were treated with different concentrations of 3-*O*-methylglucose (as indicated) for 24 h. Cells were harvested and lysed to extract protein for immunoblot assay. (D) The TXNIP reporter vectors-transfected LNCaP cells, as indicated, were treated with 1 mM 3-*O*-methylglucose (black bars) or 30 mM 3-*O*-methylglucose (white bars) for 24 h. Experimental data are presented as mean percentage ($\pm$ S.E.M.; $n=6$) of luciferase activity produced by 30 mM 3-*O*-methylglucose treatment relative to 1 mM 3-*O*-methylglucose-treated samples. (E) The TXNIP reporter vector-transfected LNCaP cells were treated with 1 or 15 mM of glucose as indicated with or without 10 mM of *N*-acetylcysteine (NAC) for 24 h. Experimental data are presented as mean percentage $\pm$ S.E.M. ($n=6$) of luciferase activity induced by different treatments relative to the 1 mM glucose treatment group. (*: $P<0.05$; **: $P<0.01$).

lipid metabolism (Hui *et al.* 2004, Schulze *et al.* 2004, Sheth *et al.* 2005, Oka *et al.* 2006). In two recent studies, oligonucleotide microarray analysis of intact human pancreatic islets and rat fibroblasts has indicated that TXNIP is upregulated by glucose (Hirota *et al.* 2002, Shalev *et al.* 2002). These studies suggest that TXNIP participates in the regulation of glucose metabolism, although the precise mechanisms are still unknown.

Increased glucose consumption is a common characteristic of malignant cells and is linked to increased energy production from aerobic glycolysis. Malignant cells exhibit altered metabolic patterns with increased reliance on anaerobic metabolism of glucose to lactic acid, even in the presence of abundant oxygen (Hochachka *et al.* 2002). Expressions of glucose transporter proteins 1 and 12 have been demonstrated in prostate cancer cells and tissues (Chandler *et al.* 2003). Other studies have also indicated that glucose uptake is increased in prostate cancer cells and that high rates of glucose consumption are required for rapid proliferation of androgen-independent prostate cancer cells (Singh *et al.* 1999). However, events involving the

regulatory mechanisms of glucose on gene profiles of the human prostate are still not well known. In the present study, glucose-upregulated TXNIP gene expression was observed both in the prostate and hepatocellular carcinoma cells (HepG2) suggesting that modulation of glucose at the TXNIP gene may persist in both metabolic and non-metabolic organs.

Metabolites clearly regulate gene transcription via metabolic pathways for carbohydrates, fatty acids, and triglycerides (Towle 1995). Results from several independent laboratories have demonstrated that glucose may induce gene expression in the glycolytic pathway as well as coordinate long-term control of the enzymes required for fatty acid and triglyceride synthesis (Vaulont *et al.* 2000, Veech 2003). Moreover, the effects of glucose regulation on gene expression are dependent on the ChoRE within the promoter of the responsible gene which is recognized by ChREBP (Rufo *et al.* 2001, Yamashita *et al.* 2001, Elsas *et al.* 2002). When the ChREBP gene is knocked-out, rat hepatocytes exhibit diminished glucose regulation of lipogenic enzymes (Ishii *et al.* 2004). Overexpressed

Figure 6 Glucose treatment induces nuclear proteins of LNCaP cell binding to the putative carbohydrate response element of TXNIP promoter. Electrophoretic mobility shift assay was performed as described in 'Materials and methods' using the 25 bp ChoRE oligonucleotide probe end-labeled with ³²P (ChoRE probe) and nuclear extract from LNCaP cells (NE) after treatment with different concentrations of glucose as indicated. Gel shift intensity decreased when the reaction mixture was challenged with unlabeled double-stranded oligonucleotide (ChoRE) containing the carbohydrate response element but not when challenged with oligonucleotide featuring a mutation at the ChREBP binding site (ChoREm).

ChREBP in primary hepatocytes induces ChoRE-containing L-type pyruvate kinase and acetyl-CoA carboxylase α activity (Yamashita *et al.* 2001, Ma *et al.* 2005). The consensus sequence of ChoRE is composed of two palindromic E-box (CACGTG) or E-box-like sequences separated by five nucleotides (Rufo *et al.* 2001, Yamashita *et al.* 2001). However, other studies have suggested that the E-boxes of ChoRE are separated by varying numbers of nucleotides (Portois *et al.* 1999, Elsas *et al.* 2002, Wang & Wollheim 2002). In this study, transient gene expression with 5'-deletion assay indicates that the effect of glucose modulation on the promoter activity of TXNIP depends on the DNA fragment located -420 to -1 bp upstream of the translational starting point of the TXNIP gene. Although two putative ChoRE sequences, -430 to -414 and -341 to -324, were observed within this region (-524 to -1), transient gene expression assays revealed that only the ChoRE mutation from CACGAGGGCAGCAGCAG to TTTGAGGGCAGCAGCAG

blocked the effects of 15 mM glucose treatment. The putative ChoRE also was confirmed by EMSA. Although a previous report (Minn *et al.* 2005) predicted the same putative ChoRE in the human TXNIP gene using *in silico* sequence analysis, the current study clearly demonstrates that the effects of glucose are mediated via a region located -341 to -324 bp upstream of the translational starting point of the TXNIP gene (Fig. 4B and C).

The E-box is also recognized by other transcription factors such as c-Myc, which is known to be required for glucose-mediated induction of metabolic enzyme genes (Osthus *et al.* 2000, Collier *et al.* 2003). The current study demonstrates that overexpression of ChREBP in prostate carcinoma cells induces activity at the ChoRE-containing TXNIP promoter in low glucose conditions. However, experimental results reveal no evidence of c-Myc participation in gene regulation of glucose on TXNIP. The TXNIP reporter vector, containing a deletion of the region -3104 to -1614 bp upstream of the translational starting point, demonstrated only 40% of the inductive response to glucose, compared with the full promoter. However, there was no consensus sequence of ChoRE found in this region (-3104 to -1614). Therefore, results suggest that other unidentified transcription factors may be involved in the modulation of glucose induction of TXNIP promoter activity.

In the present study, we demonstrate that high dose (30 mM) 3-O-methylglucose can increase TXNIP promoter activity in both LNCaP and HepG2 cells, even without the 3-O-methylglucose phosphorylation. Moreover, it seems that HepG2 cells are more sensitive to 3-O-methylglucose treatments than LNCaP cells; 15 mM 3-O-methylglucose treatment induced a twofold increase in TXNIP promoter activity in HepG2 cells. These results are similar to previous studies, which have indicated that 3-O-methylglucose induces TXNIP expression through glucose metabolism-independent pathways in human pancreatic β -cells (INS-1; Minn *et al.* 2005, 2006). Glucose and 3-O-methylglucose activation of TXNIP gene expression might be dependent on the same putative ChoRE. However, mutant ChoRE (-341 to -324) from CACGAGGGCAGCAGCAG to TTTGAGGGCAGCAGCAG only abolished 50% of the activating effects of 30 mM 3-O-methylglucose on TXNIP promoter activity (Fig. 5D). Our experiments, using mannitol to exclude an osmotic effect, suggest that unidentified factors or signals, other than ChREBP, are required for modulation of TXNIP promoter activity by 3-O-methylglucose.

Although TXNIP modulates cell proliferation by interacting with TRX, the extent to which the effect of deregulated glucose on TXNIP gene expression is dependent on the TRX in the prostate is yet unknown. Previous studies have found suppression of TXNIP expression by hydrogen peroxide is inhibited by a

cell-permeable antioxidant, *N*-acetylcysteine (Schulze *et al.* 2002, Wang *et al.* 2002). In the present study, transient gene expression assay revealed that upregulation of *TXNIP* gene expression by glucose was not inhibited by *N*-acetylcysteine suggesting that TRX may not play a pivotal role in this modulation of glucose.

This study demonstrates the expression of *TXNIP* in prostate carcinoma cells and the high metastasis rate of cells with lower *TXNIP* abundant than normal or low metastasis prostate cells. This study is the first report that demonstrates the effects of upregulated glucose on *TXNIP* gene expression in both prostate carcinoma cells and hepatocellular carcinoma cells. Glucose modulates *TXNIP* gene expression via a ChoRE in the *TXNIP* gene promoter through the ChREBP pathway, but not the c-Myc or TRX pathway.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of the research reported.

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