Supporting Information

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SI Text

Effect of IGF1 and Insulin on TXNIP Protein Expression. To test the effect of IGF1 and insulin on TXNIP protein expression, 24-h serum-starved HEK293 cells were treated with IGF1 or insulin for 5 or 12 h. Consistent with the results shown above for TXNIP mRNA, TXNIP protein levels increased in control cells upon serum starvation (Fig. S2). Both IGF1 and insulin down-regulated TXNIP protein expression at both time points. Similar to the effect at the mRNA level, IGF1 was more potent that insulin in inhibiting TXNIP protein levels.

Immunofluorescence Analysis of Oxidative Stress-Induced TXNIP Expression in Breast Cancer Cells. To further investigate the TXNIP expression and cellular localization in breast cancer cells in vitro, an immunofluorescence assay was performed. MCF7 cells, a luminal breast cancer line, were incubated with H_2O_2 (300 mM) for 4 h and then labeled with a specific TXNIP antibody conjugated with Alexa Fluor-488 conjugated goat anti-rabbit IgG secondary antibody. TXNIP labeling superimposed with nuclear labeling (DAPI) revealed that TXNIP was localized mostly in the cytoplasm of control untreated cells. Hydrogen peroxide treatment markedly enhanced TXNIP protein expression in cytoplasm as well as nucleus (Fig. S3).

Expression of TXNIP in Benign and Malignant Prostate and Breast Cancer Cells. To evaluate the hypothesis that TXNIP may exhibit a tumor suppressor role in adult epithelial tumors, basal TXNIP mRNA levels were measured in two pairs of cell lines representing benign and malignant prostate and breast cancer stages. The P69 cell line constitutes a benign prostate cancer cell line, whereas the P69-derived M12 line represents a metastatic stage of the disease. Likewise, the MCF10A cell line represents a benign breast tumor stage, whereas MCF7 cells are highly aggressive. Consistent with a putative tumor suppressor role, TXNIP mRNA levels were 75% lower in metastatic M12 cells compared with benign P69 cells. Similarly, TXNIP mRNA levels were 47% lower in malignant MCF7 cells compared with benign MCF10A cells (Fig. S5 *A* and *B*).

Effect of TXNIP on Migration Assays. Previous studies have shown an association between low TXNIP expression and tumor metastases (1). To evaluate the hypothesis that *TXNIP* constitutes a bona fide tumor suppressor gene, we overexpressed a TXNIP cDNA in androgen-independent metastatic prostate cancer-derived PC3 cells and performed migration assays. Cells were plated for 24 h and a scratch assay was performed at 80% confluence. As shown in Fig. S6, control cells covered at least 50% of the gap at 24 h and most of the damaged area by 52 h. In contrast, the scratch was still largely uncovered in TXNIP-expressing PC3 cells at this time point. These results indicate that TXNIP displays an inhibitory role on migration of metastatic cells.

SI Materials and Methods

Immunofluorescence Assays. Cells were cultured on 13-mm sterile coverslips in 24-well plates. Cells were rinsed with PBS and fixed for 30 min with 4% paraformaldehyde at room temperature, followed by permeabilization for 30 min. After overnight incubation with a rabbit polyclonal antibody against human TXNIP, the slides were incubated for 1 h with Alexa Fluor-488 conjugated goat anti-rabbit IgG secondary antibody. Coverslips were mounted in Vectashield HardSet Mounting Medium and cells were visualized using a confocal laser-scanning microscope, Leica TCS SP8.

Migration Assays. Cell migration was monitored using scratch assays. PC3 cells were seeded 24 h prescratch at a density of 20,000 cells per well in a 96-well ImageLock microplate (Essen BioScience). Scratches were done using a WoundMaker designed for these specific plates. The plates were placed into an IncuCyte ZOOM system for 72 h, with repeat scanning every 2 h. Analysis of the results was done using the IncuCyte ZOOM software.

1. Zhou J, Chng WJ (2013) Roles of thioredoxin binding protein (TXNIP) in oxidative stress, apoptosis and cancer. *Mitochondrion* 13:163–169.

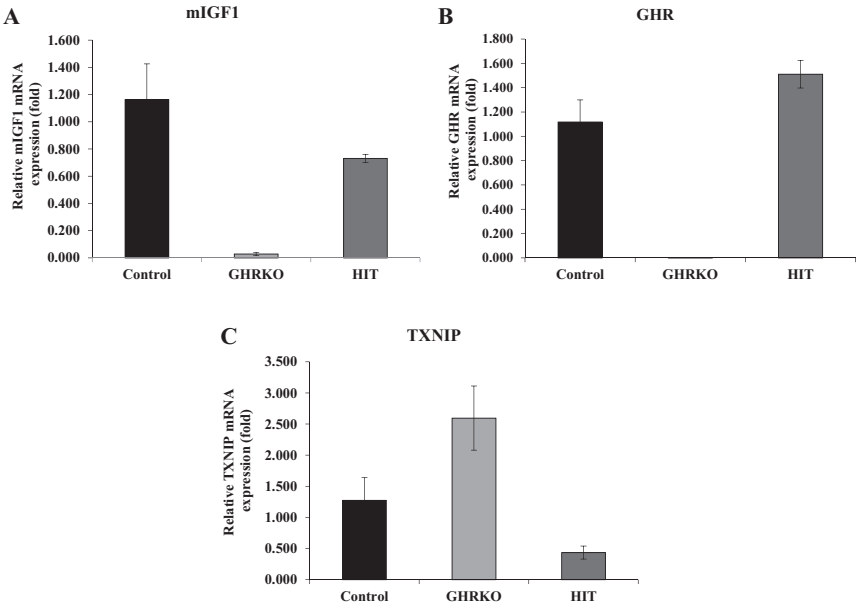

Fig. S1. TXNIP gene expression in mouse liver. RNA was extracted from livers of 4-mo-old control and GHRKO and HIT male mice, and TXNIP mRNA levels were measured by RT-QPCR. The bars represent the mean ± SEM of four mice per group. (*A*) Relative levels of mouse IGF1 mRNA expression. (*B*) Relative levels of GHR mRNA expression. (*C*) Relative levels of TXNIP mRNA expression.

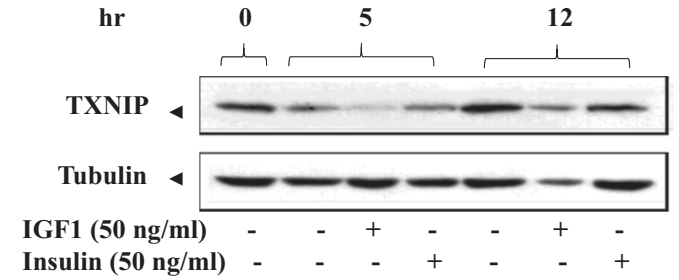

Fig. S2. Effect of IGF1 and insulin on TXNIP protein levels. Serum-starved HEK293 cells were stimulated with IGF1 or insulin for 5 or 12 h, or left unstimulated. Lysates (100 μg) were analyzed by Western blot for TXNIP and tubulin levels. Shown are the results of a typical experiment, repeated three times with similar results.

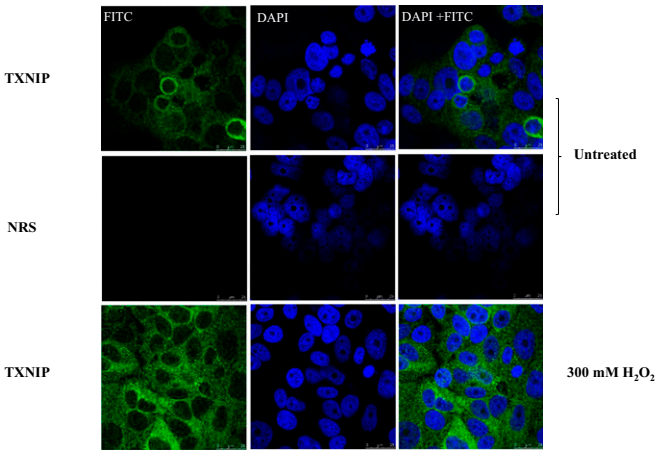

Fig. S3. Immunofluorescence analysis of oxidative stress-induced TXNIP expression in breast cancer cells. Fixed cells were treated with 300 mM of H_2O_2 for 4 h and then stained for TXNIP with fluorescent goat anti-rabbit IgG (FITC) (green) and for nucleus with DAPI (red). Merging of pictures (FITC + DAPI) gives a yellow color in the cytoplasm and nucleus. Normal rabbit serum (NRS) was used as a negative control.

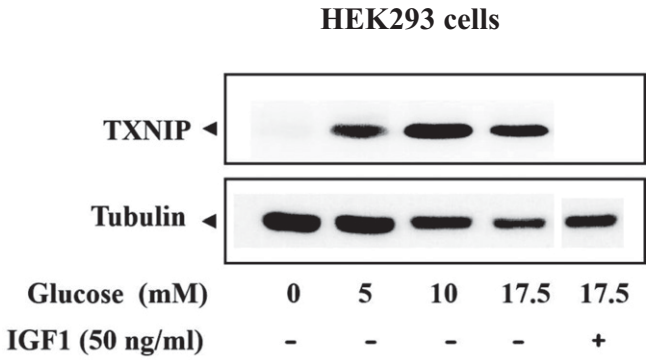

Fig. S4. Effect of IGF1 on glucose-induced TXNIP. HEK293 cells were serum deprived for 24 h, after which they were exposed to different concentrations of glucose, in the presence or absence of IGF1 for 6 h. At the end of the incubation, lysates were prepared and TXNIP and tubulin levels were measured by Western blotting.

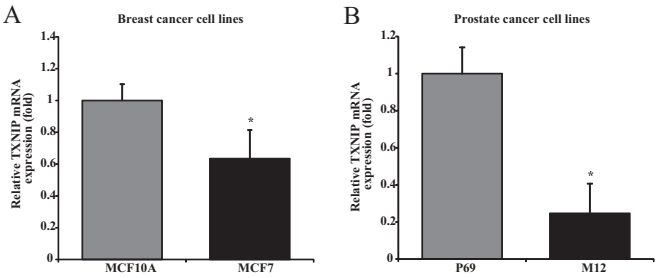

Fig. S5. Expression of TXNIP mRNA in benign and malignant (*A*) breast cancer and (*B*) prostate cancer cells. Total RNA was prepared from benign MCF10A and malignant MCF7 breast cancer cell lines, and benign P69 and metastatic M12 prostate cancer cell lines, and TXNIP mRNA levels were measured by RT-QPCR. TXNIP mRNA levels in MCF10A cells and P69 cells were arbitrarily given a value of 1. Bars are mean ± SEM of three independent measurements. *$P < 0.01$, significantly different versus MCF10A and P69 cells, respectively.

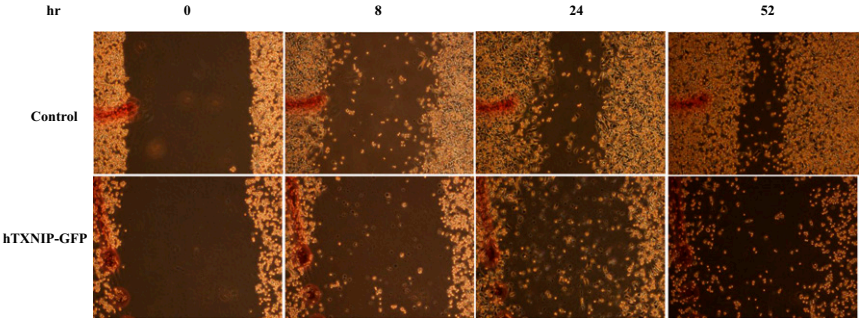

Fig. S6. Effect of TXNIP expression on migration assays. TXNIP-overexpressing and control PC3 prostate cancer cells were seeded at a density of 20,000 cells per well. Migration was evaluated using wound-healing assays as described in *Materials and Methods*. Images from the same area were captured at 0, 8, 24, and 52 h after wounding. In the control cells, the migration was statistically faster compared with TXNIP-overexpressing cells.