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Mechanistic target of rapamycin (mTOR) dependent regulation of thioredoxin interacting protein (TXNIP) transcription in hypoxia

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ABSTRACT

Thioredoxin interacting protein (TXNIP), first identified as an inhibitor of thioredoxin, is also a tumor suppressor as well as an inhibitor of lipogenesis. TXNIP is known to be transcriptionally regulated in response to nutrients such as glucose and stress signals, including endoplasmic reticulum stress and lactic acidosis. In this study, we characterized the transcriptional regulation of TXNIP in response to hypoxia. Using a hepatocellular carcinoma cell line, we have found that TXNIP mRNA expression is regulated in a biphasic manner in hypoxia whereby TXNIP expression showed an initial rapid decrease, followed by an increase under prolonged hypoxia. Interestingly, we have shown that TXNIP induction in prolonged hypoxia is independent of the Hypoxia-Inducible Factor (HIF) transcription factor. The effect of hypoxia on TXNIP expression is mediated via the inhibition of the 4E-BP1/eIF4E axis of mechanistic target of rapamycin (mTORC1). Thus, we found that inhibiting mTORC1-dependent 4E-BP1 phosphorylation mimics the effect of hypoxia on TXNIP expression. Furthermore, overexpressing eIF4E prevents the induction of TXNIP in hypoxia. Our results suggest that mTORC1 may be an important regulator of hypoxia-dependent gene expression.

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1. Introduction

Hypoxia is a type of microenvironmental stress prevalent in solid tumors, alongside with deprivation of glucose and other nutrients, as well as extracellular acidosis. To mediate adaptive responses to reduced oxygen levels, protective mechanisms at the transcriptional level are activated. These responses facilitate adjustments in cellular physiology and metabolism for survival and growth under hypoxic conditions. The major regulator of the cellular response to hypoxic stress is the transcription factor Hypoxia-Inducible Factor 1 (HIF-1). HIF-1 exists as a heterodimer of a constitutively expressed nuclear subunit, HIF-1β, and a α-subunit, HIF-1α or HIF-2α, that is controlled by cellular oxygen levels. As a result, HIF-1 transcriptional activity is regulated in an oxygen-dependent manner. In the presence of oxygen, HIF-1α is unstable and is rapidly degraded. This is due to the hydroxylation of proline residues in HIF-1α by HIF prolyl hydroxylase domain (PHD) enzymes. The hydroxylation of HIF-1α leads to the binding of the von Hippel-Lindau tumor suppressor protein (pVHL), a substrate adaptor component of an E3 ubiquitin ligase complex. Subsequent ubiquitination of the HIF-1α protein by the pVHL E3 ligase complex triggers a rapid proteasome-dependent degradation of HIF-1α [1]. Under hypoxic conditions, the activity of PHD enzymes is inhibited because in addition to 2-oxoglutarate and iron, oxygen is required as a substrate to catalyze the hydroxylation reaction. Therefore, hypoxia prevents the binding of pVHL and significantly prolongs the half-life of HIF-1α. This allows HIF-1α accumulation and translocation into the nucleus where it dimerizes with HIF-1β, followed by binding to the Hypoxia-Response Element (HRE) of hypoxia-inducible genes. In the face of oxygen deficits, HIF orchestrates cellular adaptations by inducing the expression of a wide array of genes, including pro-angiogenic factors and genes involved in glycolysis and glucose transport [2].

Thioredoxin-interacting protein (TXNIP) is an important gene that is known to be transcriptionally regulated in response to hypoxia. TXNIP, also known as Vitamin D3 up-regulated protein (VDUP1), was first identified as an endogenous inhibitor of thioredoxin [3]. TXNIP has also been demonstrated to function as an important regulator of cellular glucose [4–6] and lipid metabolism [7,8]. Furthermore, TXNIP is believed to function as a tumor suppressor gene, and is often suppressed in various human tumors. Importantly, TXNIP-deficient mice have markedly increased incidence of hepatocellular carcinoma as well a number of other cancers [6,9].

TXNIP has also been shown to be transcriptionally upregulated by glucose [10]. In addition, various types of cellular stress have been shown to cause an upregulation of TXNIP. These include oxidative damage, heat shock, UV irradiation, endoplasmic-reticulum stress, lactic acidosis and hypoxia [11–13]. TXNIP was shown to be

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a hypoxia-induced gene in human microendothelial cells [14] and in murine heart [15]. TXNIP has also been reported to be induced during hypoxia in a HIF-dependent manner in pancreatic cancer [16]. In contrast, we have recently reported that hypoxia results in a rapid decrease in TXNIP mRNA and protein expression via a HIF-independent mechanism in Hela cells [17]. Therefore, the effect of hypoxia on TXNIP expression and the role of HIF remain controversial.

Given the importance of TXNIP as a major regulator of cellular redox state and metabolism and the well-established link between TXNIP and human diseases such as diabetes and cancers, a thorough understanding of the transcriptional regulation of TXNIP in hypoxia is important. In this study, we focus on the mechanistic aspect of hypoxia-dependent regulation of TXNIP. We found that hypoxia caused a biphasic response to TXNIP expression, whereby TXNIP is initially downregulated followed by a transcriptional induction under prolonged hypoxia. Interestingly, hypoxia-dependent induction of TXNIP expression is independent of the HIF pathway. We also provide evidence that the upregulation of TXNIP under prolonged hypoxia is a consequence of the inhibition of the 4EBP1-eIF4E axis of the mTOR signaling pathway.

2. Materials and methods

2.1. Plasmid construct and transfection

pcDNA3-HA-eIF4E was a gift from Dong-Er Zhang (Addgene plasmid 17343) [18]. HEK293T cells were transfected using GeneJuice® Transfection Agent (Novagen) according to the manufacturer's instructions.

2.2. RNA extraction and real-time quantitative PCR

Following various treatments, cells were subjected to total RNA extraction using TRIzol (Invitrogen). Real-time quantitative PCR of the mRNA was carried out using iScript™ One-Step RT-PCR Kit with SYBR® Green (Bio-Rad). Real-time PCR analysis was performed the iQ5 Optical System Software Version 2.0 (Bio-Rad). The following specific primers were used for real-time PCR, human TXNIP (F: 5′-GGCGGGTGTCTGTCTCTGCT-3′, R: 5′-GGCAAGGTAAGT GTGGCGGG-3′); human β-actin (F: 5′-GCCGACAGGATGCAGAAG-GAGATCA-3′, R: 5′AAGCATTTGCGGTGGACGATGGA-3′); mouse TXNIP (F: 5′-GAAGTTACCCGAGTCAAAGC-3′, R: 5′-CGCAAGTAGTCCAAAG TCTG); mouse β-actin (F: 5′-CCTGACGGCCAGGTCATC-3′, R: 5′-GACAGCACTGTGTTGGCATAGAG-3′). Values are expressed as relative expression of mRNA normalized to β-actin mRNA. Results represent the averages ± SD of the triplicates from two independent experiments and are presented as fold induction compared to normoxic conditions. One-way ANOVA was used to analyze statistical significance. Two-tailed $P < 0.05$ was considered significant.

2.3. Western blot analysis

Whole cell extracts were lysed in Triton lysis buffer as previously described [17]. The following antibodies were used: mouse monoclonal anti-human TXNIP/VDUP-1 (MBL International), rabbit anti-phospho-p70S6 kinase (Thr389) (Cell Signaling), rabbit anti-p70S6 kinase (Cell Signaling), rabbit Phospho-4E-BP1 (Thr37/46) (236B4), rabbit 4E-BP1 (53H11), mouse monoclonal anti-human β-actin (Sigma–Aldrich).

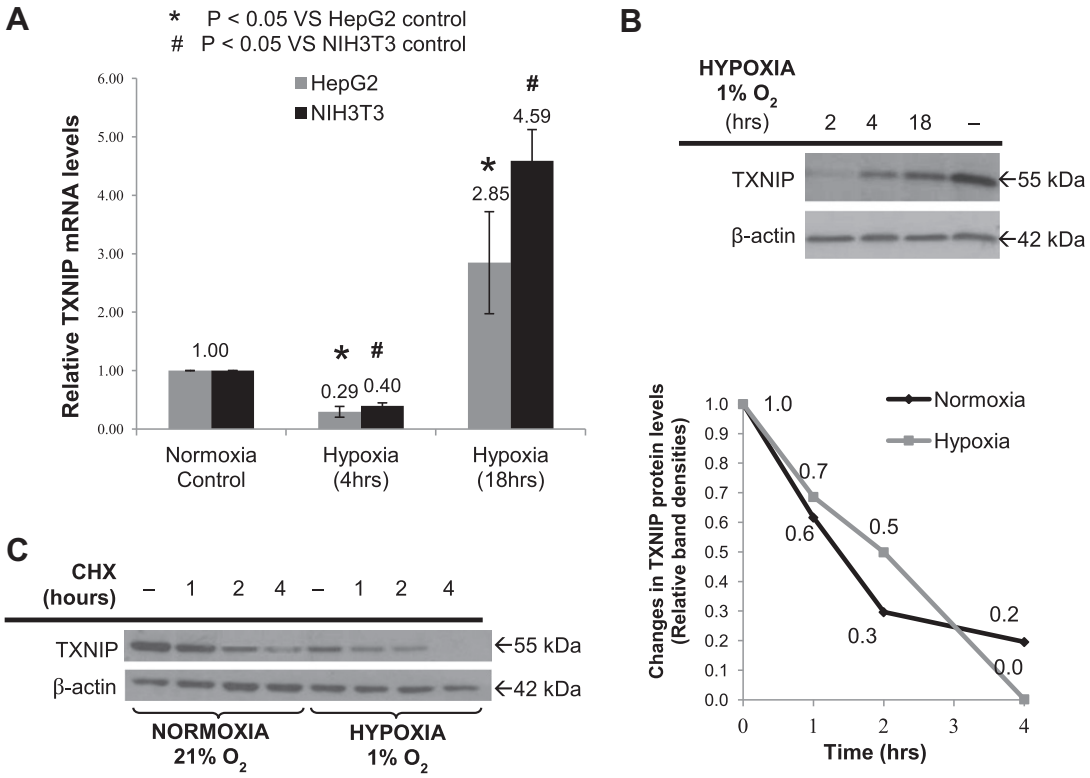

Fig. 1. Hypoxia regulates TXNIP in a biphasic manner. (A) Measurement of TXNIP mRNA levels in hypoxia HepG2 and NIH3T3 cells were incubated in hypoxia (1% O_2) for 4 h and 18 h as previously described [17]. TXNIP mRNA levels were measured using real-time PCR. Values are expressed as average ± SD, normalized to β-actin mRNA. Treatment under normoxia (21% O_2) is set as 1-fold ($^{\#/*}P < 0.05$). (B) Changes in TXNIP protein in hypoxia Cell lysates of HepG2 cells were collected after 2, 4 and 18 h of hypoxia (1% O_2) incubation, followed by Western blotting using TXNIP antibody. β-Actin was used as loading control. (C) Western blot analysis of TXNIP protein half-life in normoxia and hypoxia. HepG2 cells were first pre-incubated in normoxia or hypoxia for 18 h. Cell lysates were collected at 0, 1, 2 and 4 h after addition of 40 μM cycloheximide (CHX). The half-life of TXNIP in normoxia and hypoxia was monitored using Western blot. (Left panel) Densitometric analysis of Western blot was performed with the use of imageJ. Results are presented as relative optical density of TXNIP normalized to that of the loading control, β-actin. (Right panel).

2.4. Luciferase reporter assay

HepG2 cells were seeded in 12-well plates and transfected with pGL3-HRE-Luc for 24 h. Hypoxic treatment of cells was carried out for the last six hours before cell lysis using the Steady-Glo® Luciferase Assay System (Promega) and the luciferase activity was measured using a 20/20n Luminometer (Turner Biosystems). Data are presented as the average ± SD of a representative experiment performed in duplicates. One-way ANOVA was used to analyze statistical significance. Two-tailed $P < 0.05$ was considered significant.

2.5. siRNA silencing

HIF-1β siRNA were obtained from Integrated DNA Technologies (IDT). The following oligo sequences were used for the siRNA knockdown; HIF-1β siRNA #1 (5′-GCAAACAGAAUUGGACAUGGUACCA-3′, 5′-UGGUACCAUGUCCAAUUCUGCUUGCUG-3′); HIF-1β siRNA #2 (5′-CAAUGCG GAUCAGAGUAAAGGCATC-3′, 5′-GAUGCCUUUACUCUGAUCCGCAUUGUU-3′). Transfections of HIF-1β siRNA were performed using Lipofectamine Plus (Invitrogen), according to manufacturer's instructions.

3. Results

3.1. Hypoxia regulates TXNIP in a biphasic manner

To address the controversies surrounding the effect of hypoxia on the expression of TXNIP, we studied the effect of short and long term hypoxia on TXNIP expression. To determine the effect of hypoxia on TXNIP mRNA levels, HepG2 and NIH3T3 cells were incubated under hypoxia for 4 and 18 h. mRNA extraction, followed by quantification of TXNIP mRNA was carried out using real-time PCR. A biphasic pattern in the mRNA levels was observed during the two indicated time points of hypoxia in both cell lines (Fig. 1A) The TXNIP mRNA levels initially decreased after 4 h of hypoxia, followed by an upregulation after 18 h of hypoxic incubation. We have previously observed a similar biphasic response in TXNIP expression under hypoxia in Hela cells [17]. In view of the mRNA findings, we also examined the changes in endogenous TXNIP protein levels after 2, 4 and 18 h of incubation under hypoxia in HepG2 cells using Western Blot analysis. A dramatic decrease in TXNIP protein expression was observed after 2 h hypoxic treatment as compared to the normoxia control (Fig. 1B). Subsequently, a gradual increase in TXNIP level was observed under prolonged hypoxia, albeit levels did not increase above the basal TXNIP expression under normoxia (Fig. 1B). The biphasic response of TXNIP mRNA and protein expression in hypoxia may explain the contradicting findings with regards to the effect of hypoxia on TXNIP expression [14–17].

A potential explanation for the less pronounced increase in TXNIP protein compared to mRNA levels under prolonged hypoxia may be a faster rate of TXNIP protein degradation. To monitor the rate of TXNIP protein degradation, a cycloheximide (CHX) pulse-chase experiment was performed in both normoxic and hypoxic conditions. The degradation rate of TXNIP in normoxia was comparable to that in hypoxic conditions (Fig. 1C). Thus, a decrease in protein stability is unlikely the reason for the discrepancy between TXNIP mRNA and protein expression during prolonged hypoxia.

3.2. Effect of prolyl hydroxylase domain (PHD) enzyme inhibitors on TXNIP expression

PHD-catalyzed hydroxylation reactions are dependent on oxygen concentrations and these enzymes serve as cellular oxygen sensors. Under hypoxic conditions, the oxygen-sensing PHDs can no longer function. Therefore, we hypothesized that the TXNIP expression observed under hypoxic conditions could be due to hypoxia-mediated inhibition of PHD enzymes. To investigate this

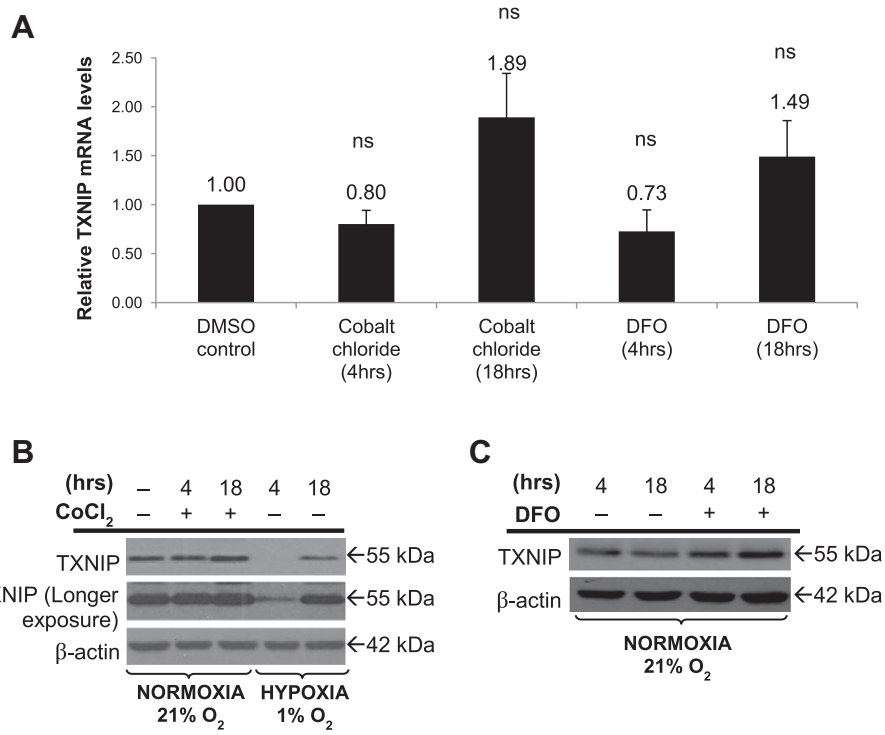

Fig. 2. Effect of prolyl hydroxylase domain (PHD) enzyme inhibitors on TXNIP expression. (A) Measurement of TXNIP mRNA levels upon treatment with PHD enzymes inhibitors TXNIP mRNA was measured by real-time RT-PCR in HepG2 cells after 18 h of drug treatment with 0.2 mM desferrioxamine (DFO) and 0.1 mM cobalt chloride ($CoCl_2$) under normoxia (21% O_2) (*$P < 0.05$, ns: not significants). (B and C) Changes in TXNIP protein upon treatment with PHD enzymes inhibitors HepG2 cells were treated with (B) 0.1 mM $CoCl_2$ and (C) 0.2 mM DFO under normoxia (21% O_2) for 4 h and 18 h. Cell lysates were collected for Western blotting analysis using TXNIP antibody. β-Actin was used as loading control.

possibility, we treated HepG2 cells with two different PHD enzyme inhibitors, cobalt chloride ($CoCl_2$) and desferrioxamine (DFO) and monitored the changes in the TXNIP mRNA and protein levels. $CoCl_2$ and DFO inhibit the catalytic function of PHD enzymes by chelating of or competing with intracellular iron, a cofactor required for the hydroxylation reaction. These PHD enzyme inhibitors caused a slight decrease in TXNIP mRNA expression after 4 h treatment (Fig. 2A). However, this decrease in TXNIP mRNA expression was less pronounced as compared to the effects of hypoxia. Furthermore, treatment with these inhibitors did not result in a change in TXNIP protein levels (Fig. 2B and C). In contrast, 4 h of hypoxia caused a dramatic decrease in TXNIP protein expression (Fig. 2B). Under prolonged treatment with the PHD enzyme inhibitors, we observed an upregulation of TXNIP mRNA expression in HepG2 cells (Fig. 2A). However, the degree of upregulation of TXNIP mRNA was smaller compared to that under prolonged hypoxia (Compare Figs. 1A and 2A). Furthermore, in hypoxia, there was a dramatic upregulation of TXNIP protein concentration from 4 to 18 h (Figs. 1B and 2B). In contrast, we did not observe any change in TXNIP protein expression in $CoCl_2$ and DFO treated cells during the same time period (Fig. 2B and C). Taken together, these results suggest that the regulation of TXNIP in hypoxia may be partially dependent on PHD enzymes. Given that the major role of PHD enzymes is to regulate the stability of the HIF-α transcription factor in response to oxygen availability, we next investigate if TXNIP expression is regulated via the HIF transcriptional pathway under hypoxic conditions.

3.3. Long-term hypoxia regulates TXNIP expression in a HIF-independent manner

Given that the activation of many oxygen-regulated genes is mediated by HIF, the regulation of TXNIP expression in hypoxia may be HIF-dependent. We have previously shown that the down-regulation of TXNIP under acute hypoxia is HIF-independent. To examine the role of HIF under prolonged hypoxia in HepG2 cells, we used an siRNA based approach. HIF-1β was chosen as the knockdown candidate as both HIF-1α and HIF-2α are present in HepG2 cells and hetero-dimerization of either HIF-1α or HIF-2α with HIF-1β can lead to HIF transcriptional activity [19]. Therefore, siRNA mediated deletion of HIF-1β would prevent both HIF-1α and HIF-2α dependent HIF transcriptional activity. To validate the efficiency of the HIF-1β knockdown, HepG2 cells were transfected

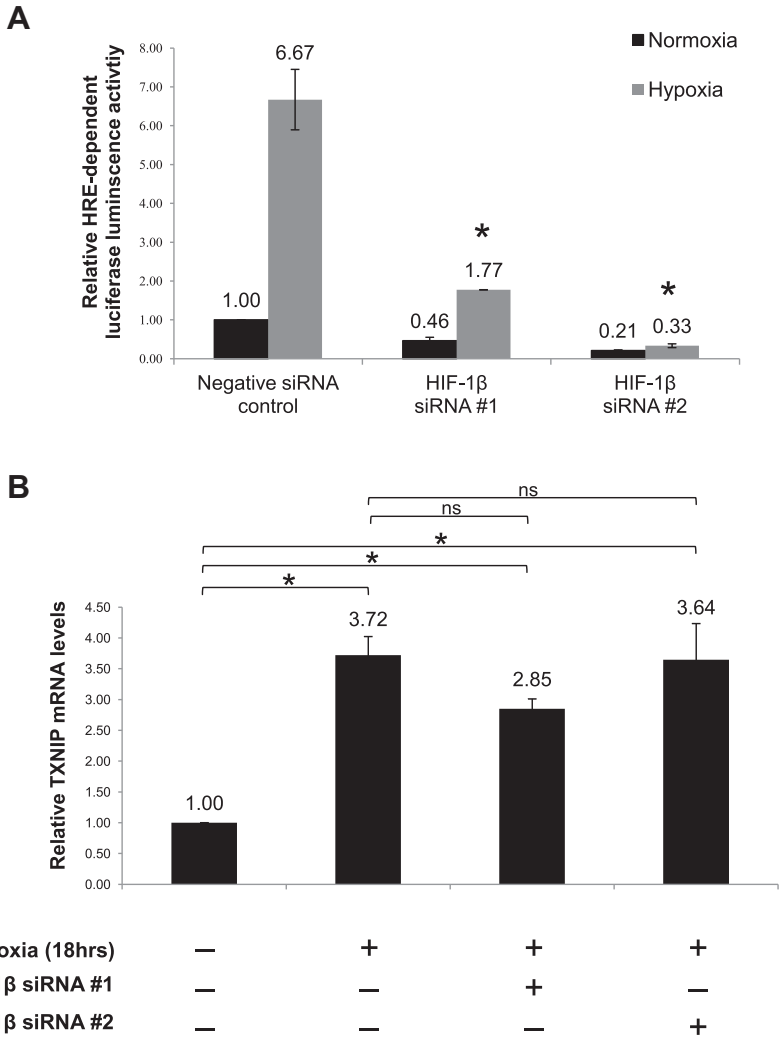

Fig. 3. Long-term hypoxia regulates TXNIP expression in a HIF-independent manner. (A) Validation of HIF-1β knockdown using HRE-dependent luciferase reporter activity HepG2 cells were transfected with two different HIF-1β siRNA oligonucleotides and 24 h later with a HRE-dependent luciferase reporter plasmid. 24 h post-transfection, HRE-luciferase luminescence measurements were taken after 6 h of hypoxia (1% O_2) incubation. Data are presented as the average ± SD of a representative experiment performed in duplicates (one-way ANOVA, $^*P < 0.05$) (B) Measurement of TXNIP mRNA levels in HIF-1β KO cells HepG2 cells were transfected with HIF1-β siRNA for 2 days. TXNIP mRNA expression was determined following a 18 h hypoxia incubation ($^*P < 0.05$, ns: not significants).

with two different HIF-1β siRNA oligonucleotides and 24 h later with a HRE-dependent reporter plasmid. Both oligonucleotides prevented the hypoxia-induced increase in e HRE-dependent luciferase activity, indicating a reduced HIF-transcriptional activity in HIF-1β knockdown cells (Fig. 3A).

We then determined the role of HIF in TXNIP mRNA expression in prolonged hypoxia in HepG2 cells transfected with negative control or HIF-1β siRNAs. A marked increase of 3.72 ± 0.30-fold in TXNIP mRNA for control cells grown under 18 h of hypoxic conditions relative to normoxic levels was observed (Fig. 3B). This data is consistent with the previous experiment (Fig. 1A). In cells transfected with HIF-1β siRNAs, the levels of TXNIP mRNA increased to a similar degree as compared to the control cells (2.85 ± 0.16 and 3.64 ± 0.59-fold in cells transfected with HIF-1β siRNA#1 and #2, respectively) (Fig. 3B). This strongly suggests that the upregulation of TXNIP mRNA under prolonged hypoxia is independent of the HIF transcription factor.

3.4. TXNIP upregulation under prolonged hypoxia is dependent on hypoxia–mediated inhibition of 4E-BP1

Apart from HIF, a further mediator that is highly responsive to changes in the oxygen concentration is the mechanistic target of rapamycin complex 1 (mTORC1). In the presence of oxygen, growth factors and nutrients, mTOR promotes protein synthesis and cell growth by phosphorylating two major effectors: eukaryotic initiation factor 4E (eIF4E) binding protein 1(4E-BP1) and p70S6 kinase (p70S6K). Hypoxia has been reported to cause a marked inhibition of mTORC1 [20]. The inhibition of mTORC1 in hypoxia results in a decrease in protein synthesis, a highly energy consuming process. This aids in preserving cellular ATP levels under conditions of compromised oxidative phosphorylation under hypoxic stress. Given the link between hypoxia and mTOR inhibition, we next tested if inhibition of the mTOR pathway affects the expression of TXNIP. To this end, two different mTOR

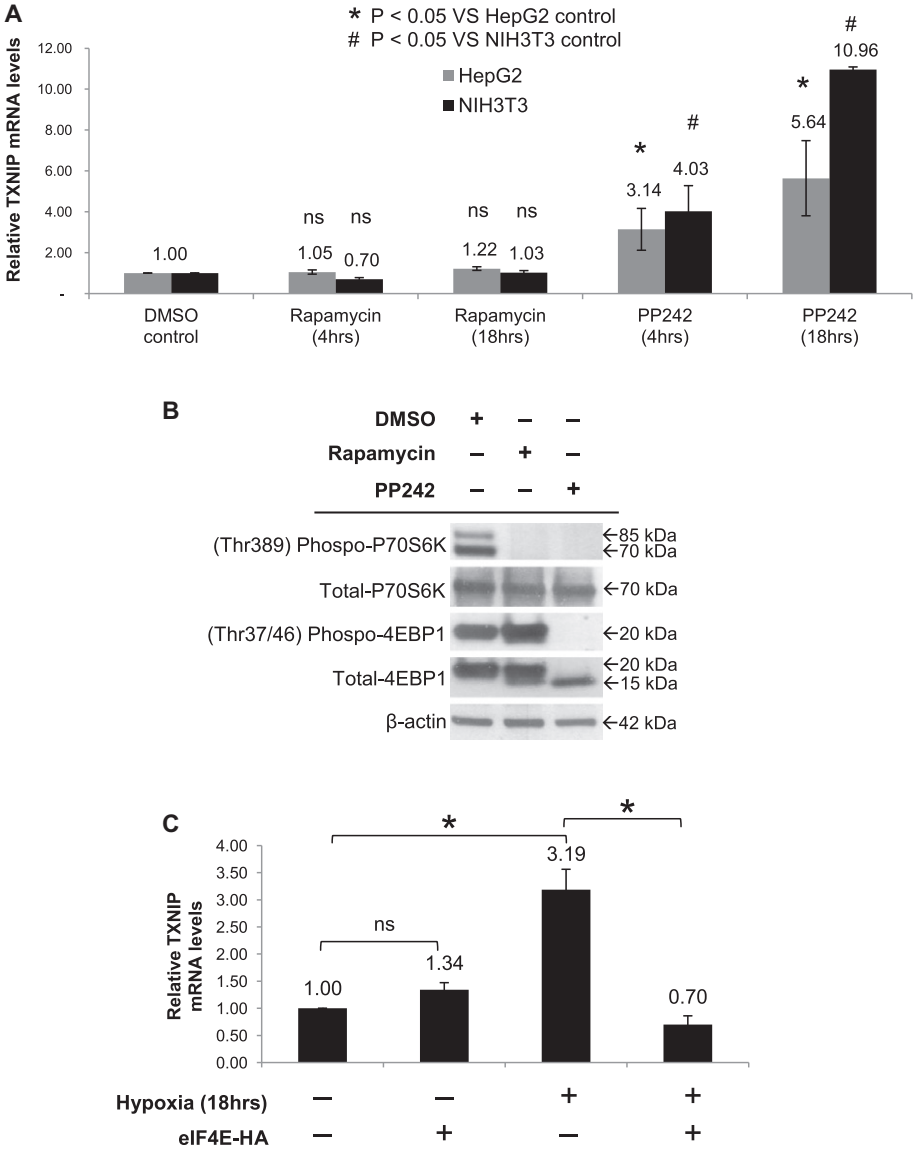

Fig. 4. TXNIP upregulation under prolonged hypoxia is dependent on hypoxia-mediated inhibition of 4E-BP1. (A) Measurement of TXNIP mRNA upon treatment with mTOR inhibitors TXNIP mRNA levels were analyzed by real-time PCR after 18 h of drug treatment with 20 nM rapamycin and 2 μM PP242. (B) Phosphospecific immunoblotting of downstream mTOR effectors HepG2 cell lysates were collected for immunoblotting after 18 h of 20 nM rapamycin and 2 μM PP242 drug treatment. β-actin was used as loading control. (C) Measurement of TXNIP mRNA in eIF4E overexpressed HEK293T cells HEK293T cells were transfected with pcDNA3-HA-eIF4E. 48 h post-transfection, cells were incubated in normoxia (21% O_2) or hypoxia (1% O_2) for 18 h, after which TXNIP mRNA expression was determined using real-time PCR. Data are expressed as normalized ratios (*$P < 0.05$, ns, not significant).

inhibitors, rapamycin and PP242, were used. Rapamycin treatment had no effect on TXNIP mRNA levels (Fig. 4A). In contrast, in PP242-treated HepG2 and NIH3T3 cells, a gradual upregulation of TXNIP mRNA was observed after 4 and 18 h of drug treatment (Fig. 4A).

Previous reports have shown that rapamycin, an allosteric inhibitor of mTORC1, inhibits only mTORC1-dependent p70S6K phosphorylation but has no effect on the phosphorylation of 4E-BP1 by mTORC1. On the other hand, PP242, an active-site mTOR inhibitor blocks phosphorylation of both downstream effectors of mTORC1 [21]. In HepG2 cells, we confirmed by phosphospecific immunoblotting of p70S6K and 4E-BP1 that both rapamycin and PP242 inhibited the phosphorylation of p70S6K on Thr389, but only PP242 inhibited mTORC1 phosphorylation of 4E-BP1 on Thr37/46 (Fig. 4B). Given that TXNIP expression was only induced by PP242 and not by rapamycin, our result suggests that TXNIP transcription is not regulated through p70S6K but via the 4EBP-1/eIF4E axis of the mTORC1 signaling pathway. This is consistent with a recent study by Wang et al. who reported that in NIH3T3 cells, TXNIP mRNA was differentially upregulated by PP242, but not by rapamycin [22].

Our results presented so far have demonstrated that both prolonged hypoxia and inhibition of mTOR lead to an upregulation of TXNIP mRNA levels (Figs. 1A and 4A). This suggests that hypoxia-induced upregulation of TXNIP transcripts is due to mTOR inhibition. Given that only PP242, but not rapamycin, increased TXNIP expression, we hypothesized that hypoxia regulates TXNIP through hypophosphorylation of 4E-BP1. In its hypophosphorylated state, 4E-BP1 inhibits cap-dependent translation by binding and inactivating the translation initiation factor eIF4E. To investigate if hypoxia-induced upregulation of TXNIP mRNA is dependent on the binding of 4E-BP1 to the eIF4E protein, eIF4E was overexpressed in HEK293T cells. TXNIP mRNA levels were measured using real-time PCR. Overexpression of eIF4E indeed prevented the upregulation of TXNIP mRNA levels under hypoxia (Fig. 4C). This result strongly suggests that TXNIP upregulation under prolonged hypoxia is dependent on hypoxia-mediated hypophosphorylation of 4E-BP1 and sequestration of eIF4E.

4. Discussion

TXNIP is a multifunctional protein and plays a role in the regulation of the cellular redox state, of glucose and lipid metabolism, inflammation and adipogenesis [23]. Hence, it is important to understand how TXNIP gene expression is controlled. It has been shown that TXNIP is highly regulated at the transcriptional level in response to various signals, including glucose levels, insulin and growth factors, lactic acidosis, energy depletion, adenosine containing compounds and endoplasmic reticulum stress [10–13]. In this study, we focus on the regulation of TXNIP transcription in hypoxia. We have shown that TXNIP displays a biphasic response to hypoxic incubation. As a rapid response to hypoxia, TXNIP mRNA and protein expression is down-regulated. Given the role of TXNIP as an inhibitor of glucose utilization, the initial TXNIP downregulation in hypoxia may serve as an adaptive mechanism to increase glucose uptake under conditions of compromised oxidative phosphorylation. During prolonged hypoxia, inhibition of TXNIP mRNA and protein expression is reversed and TXNIP mRNA levels increase above the basal concentrations in hypoxia. The induction of TXNIP protein under extended hypoxia is less pronounced compared to the increase in mRNA levels. We have shown that the degradation rate of TXNIP is comparable in normoxia and hypoxia. Hence, altered protein stability is unlikely the reason for the discrepancy between TXNIP mRNA and protein expression during prolonged hypoxia. Instead, general inhibition of protein synthesis in hypoxia at low oxygen concentrations may explain the lower induction of TXNIP protein in hypoxia.

In further studies, we elucidated the mechanism through which long term hypoxia increases TXNIP expression. The effect of hypoxia on the expression of TNXIP was partially mimicked by the PHD enzyme inhibitors $CoCl_2$ and DFO. Given the major role of PHD enzymes as important regulators of HIFα stability, we next hypothesized that TXNIP expression in hypoxia may be dependent on HIF. Based on siRNA-mediation deletion of HIF-1β, our findings demonstrated that HIF is not required for the regulation of TXNIP in hypoxia. These finding are surprising, given the important role of HIF as a master regulator of the cellular hypoxic response and as mediator of cellular oxygen homeostasis. This indicates that cells are able to sense and respond to the alterations in oxygen availability in a HIF-independent manner. However, in contradiction with our findings, TXNIP was previously reported to be a HIF-1α induced gene [16]. In this study, the authors used pancreatic cells, and hence it is possible that there are cell type specific effects of HIF-1α on TXNIP expression.

Considering other potential mechanisms through which hypoxia may affect TXNIP expression, we hypothesized that the mTORC1 signaling pathway may be involved. Various studies have reported that hypoxia can cause a marked inhibition of mTORC1 signaling [20]. Our results show that the upregulation of TXNIP under prolonged hypoxia is a result of the inhibition of the 4E-BP1/eIF4E axis of the mTORC1 signaling pathway. Thus, by using an ATP-competitive inhibitor of mTOR, we were able to mimic the effects of hypoxia on TXNIP expression. Conversely, overexpression of eIF4E prevented the upregulation of TXNIP in hypoxia. These results highlight that mTORC1 may be an important cellular regulator of oxygen-dependent gene expression and may play a role in mediating the adaptation of cells to hypoxic conditions.

It has been recently recognized that mTORC1 is involved in the stimulation of lipid synthesis [22]. Notably, a number of studies have highlighted TXNIP as a negative regulator of lipogenesis. A nonsense TXNIP mutation in mice has been shown to be associated with marked hypertriglyceridemia and hypercholesterolemia [7]. Furthermore, TXNIP knockout mice have been shown to be hyperlipidemic [8]. These findings suggest that TXNIP may be involved in mTORC1-mediated control of lipogenesis. mTORC1 also promotes adipogenesis by activating sterol regulatory element binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptor-γ (PPAR-γ) and its coactivator, Lipin1 [24,25]. Interestingly, TXNIP has recently been shown to function as an inhibitor of adipogenesis [26]. Hence, mTORC1-dependent regulation of TXNIP is likely to contribute to the regulation of adipogenesis. Our results suggest that mTORC1-dependent upregulation and maintenance of cellular TXNIP expression plays an important role in the adaption of cells to hypoxia. The exact mechanism through which TXNIP promotes hypoxic adaption is currently not clear. It is expected that a better understanding of the molecular function of TXNIP will provide more insights into its role in hypoxia. Notably, apart from inhibiting thioredoxin, the exact targets and mechanisms through which TXNIP exerts its physiological effects are currently not known and require further studies.

In summary, we have demonstrated that hypoxia play an important role in regulating the transcription of TXNIP. Interestingly, the regulation of TXNIP in hypoxia is via a HIF-independent manner but is mediated via the inhibition of 4E-BP1/eIF4E axis of mTORC1. Our results suggest that mTORC1 may be an important regulator of hypoxia-dependent gene expression.

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