Araştırma Makalesi / *Research Article*
Biyoloji / *Biology*

Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 9(1): 310-320, 2019
***Journal of the Institute of Science and Technology*, 9(1): 310-320, 2019**

DOI: 10.21597/jist.446068 **ISSN: 2146-0574, eISSN: 2536-4618**

A549 Akciğer Kanseri Hücre Hattında TIGAR'ın Susturulması, NF-κB ve HO-1 Ekspresyonlarının Modülasyonu ile Apoptozis ve Otofaji İndükler

Can Ali AĞCA[1*], Osama Hamid SHAREEF[1]

ÖZET: TP53 kaynaklı glikoliz ve apoptozis düzenleyici (TIGAR) protein, glikoz metabolizması sırasında Fru-2, 6-P2 seviyelerini glukoz metabolizması sırasında kontrol eder ve nikotinamid adenin dinükleotit fosfat (NADPH) düzeyini devam ettirerek hücre içi anahtar bir antioksidan olan glutatyonun (GSH) geri dönüştürmesine yardımcı olur. Bu çalışma, A549 hücre hattında TIGAR'ın susturulmasının altında yatan, reaktif oksijen türleri (ROS) aracılı apoptotik ve otofajik mekanizmaları araştırmak için tasarlanmıştır. siRNA-TIGAR'ın A549 akciğer kanseri hücreleri üzerindeki etkisini saptamak için hücre canlılığı, koloni oluşumu, ROS ve NADPH analizlerini gerçekleştirdik. Ek olarak, protein ve mRNA ekspresyon seviyeleri sırası ile Western blot ve Real-time PCR yöntemleri ile belirlendi. TIGAR'ın A549 hücre hattında susturulmasının ardından, çeşitli parametreler analiz edildi ve TIGAR'ın down regülasyonunun hücre canlılığını inhibe ettiği ve koloni oluşumunu azalttığı gösterildi. TIGAR'ın susturulmasının apoptozis ve otofajiyi tetiklediğini ve bunu Nükleer faktör-kappa B (NF-κB) ve Hem oksijenaz-1 (HO-1)'in indüksiyonun izlediği belirledik. Dahası, artmış ROS düzeyi ve azalmış NADPH seviyeleri gözlemlendik. Bu çalışma, akciğer kanseri hücrelerinde, NF-κB ve HO-1 ekspresyonları ile apoptozis ve otofajiyi arttırmak için TIGAR susturulmasının kullanılmasını desteklemekte ve akciğer kanserinin tedavisi için potansiyel bir hedef olarak TIGAR önermektedir.

Anahtar kelimeler: TIGAR, Akciğer kanseri, Apoptozis, Otofaji, NF-κB, HO-1

Knockdown of TIGAR Induces Apoptosis and Autophagy with Modulates NF-κB and HO-1 Expression in A549 Lung Cancer Cells

ABSTRACT: The tp53-induced glycolysis and apoptosis regulator (TIGAR) protein controls fructose-2, 6-bisphosphate (Fru-2, 6-P2) levels during glucose metabolism and helps maintain nicotinamide adenine dinucleotide phosphate (NADPH) levels to recycle glutathione (GSH), a key intracellular antioxidant. The present study was designed to investigate the apoptosis and autophagy mechanisms via reactive oxygen species (ROS) that underlie TIGAR knockdown in the A549 cell line. To detect the influence of siRNA-TIGAR on A549 lung cancer cells, we performed cell viabilty, colony formation, ROS, and NADPH assays. In addition, Western blotting and real-time polymerase chain reaction (PCR) assays were used to measure protein and mRNA expression levels, respectively. After TIGAR knockdown in A549 cell lines, various assay parameters were analyzed and showed that down-regulation of TIGAR inhibited viability and decreased colony formation. We also demonstrated that TIGAR knockdown induced apoptosis and autophagy, followed by an induction of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and heme oxygenase-1 (HO-1) expression. Furthermore, increased ROS levels and decreased NADPH levels were observed. This study supports our understanding of the possibility of employing TIGAR knockdown in lung cancer cells to enhance apoptosis and autophagy with NF-κB and HO-1 expression and then suggest TIGAR as a potential target for the treatment of lung cancer.

Keywords: TIGAR, Lung cancer, Apoptosis, Autophagy, NF-κB, HO-1

[1] Can Ali AĞCA (**Orcid ID:** 0000-0002-0244-3767), Department of Molecular Biology and Genetics, Faculty of Science and Literature, Bingol University, Bingöl, Turkey
[2] Osama Hamid SHAREEF (**Orcid ID:** 0000-0001-6859-4317), Department of Medical Laboratory Technique, Halabja Technical Institute, Sulaimani Polytechnic University, Sulaymaniyah, Iraq
*Sorumlu yazar/Corresponding Author: Can Ali AĞCA, caagca@bingol.edu.tr
.

Geliş tarihi / *Received:*19.07.2018
Kabul tarihi / *Accepted:*15.10.2018

INTRODUCTION

Lung cancer is one of the most common malignancies, and remains the leading cause of cancer-related deaths (Leung et al., 2014; Zhao et al., 2017a). When compared to other types of cancer, lung cancer rates are rapidly increasing owing to tobacco use, exposure to second-hand smoke, air pollution, and other possible causes (Bai et al., 2017; Zhao et al., 2017b). Today, advanced and effective treatment strategies are available for non-small-cell lung carcinoma (NSCLC). However, prognosis and survival rates still pose a significant challenge for treatment in clinical settings and human NSCLC cell lines. TP53-induced glycolysis and apoptosis regulator (TIGAR) is a protein that controls fructose 2, 6-bisphosphate (Fru-2, 6-P2) levels during glucose metabolism and helps maintain nicotinamide adenine dinucleotide phosphate (NADPH) levels to recycle glutathione (GSH), a key intracellular antioxidant. Silencing of TIGAR leads to increased levels of reactive oxygen species (ROS), correlating with greater resistance to cell death. This occurs by enhancing the pentose phosphate pathway (PPP), which directly contributes to deoxyribonucleic acid (DNA) repair based on generation of ribose-5-phosphate and NADPH, two key precursors of DNA repair and synthesis (Bensaad et al., 2006; Bensaad et al., 2009; Pena-Rico et al., 2011; Lee et al., 2014; Cheung et al., 2016). TIGAR functions to limit ROS in most cells; its expression has been found to be strongly associated with protection against ROS-induced cell death (Lee et al., 2014). ROS includes a range of oxygen-containing reactive chemical species, such as free radicals, hydrogen peroxide (H_2O_2) and oxygen anions. These molecules are generally highly reactive, small and short-lived (Rahal et al., 2014; Li et al., 2015). ROS production in cells has two sources: first, NADPH oxidase (NOX), which yields active superoxide through the membranes of phagosomes and neutrophils, and the second is mitochondria that produce ROS as a by-product of respiration (Li et al., 2015). The main catabolic mechanism which demonstrates connections with the phenomena taking place in cancerous cells is autophagy. It is highly regulated process controlled by certain autophagy-related genes (ATGs). This important pathway occurs in response to intra- or extra-cellular stress and may end up with continuation of cell survival. Yet, when over-activated, autophagy leads to death of cell (Wang et al., 2011). ROS can damage and oxidize products of the mitochondria, such as nucleic acids, amino acid precursors, and lipids. This leads to induction of mitochondrial dysfunction that could be triggered as a condition known as oxidative stress (Scherz-Shouval and Elazar, 2007; Li et al., 2015). Normally, sufficient ROS levels function as signals to promote cell viability and survival, while increasing ROS can induce cell death. Under certain physiological conditions, the balance between elimination and generation of ROS ensure the proper function of cells. According to previous studies, expression of both oxidative stress markers (NF-κB and HO-1) is linked to ROS levels. The transcription factor, NF-κB, plays a major role in cellular viability and apoptosis; known to be sensitive to cellular oxidative status and contribute to cell death, it may be activated by H_2O_2 (Gloire et al., 2006; Wang et al., 2007, Morgan and Liu, 2011). Additionally, ROS-induced expression of HO-1 and the mechanism of action of HO-1 in mitochondrial oxidative stress have been observed to mediate apoptotic tissue injury (Han et al., 2009; Bindu et al., 2011, Bansal et al., 2014). There is a previous study that showed the contribution of autophagy to anticancer drug-mediated antitumor activity both in vitro and in vivo, for example, in hepatocellular carcinoma

(HCC) and glioblastoma. This study investigates the effect of TIGAR siRNA on cell viability and programmed cell death in A459 lung cancer cells and analyses its potential utility as a therapeutic enhancer. We hypothesize that the balance between cell growth and ROS levels will be affected by the silencing of TIGAR. Our results indicate that siTIGAR may act as an anti-cancer molecule by decreasing the cellular growth rate and increasing ROS levels and then induce NF-κB and HO-1 markers.

MATERIALS AND METHODS

Cell Culture

Human A549 lung cancer cells were supplied by Bingol University Cancer Research Centre (BUCR). Cells were cultured in RPMI medium 1640 complete media containing 10% fetal bovine serum and 1% penicillin (1000 IU mL^{-1})-streptomycin (10 g m^{-1}) solution in a humidified 5% CO_2 incubator at 37 C°.

Transfection with small interfering RNAs (siRNA)

siRNA TIGAR, transfection medium, and transfection reagent were purchased from Santa Cruz Biotechnology Company (USA). Untreated A549 cells, growing exponentially, were plated in 6-well plates (Sigma-Aldrich, USA) at a density of 1×10^5 cells and allowed to attach overnight in a 5% CO_2 incubator set to 37 C^o. Once cells surpassed 50% confluency, transfection was performed according to the manufacturer's instructions. Briefly, the transfection mixture (30 nM siTIGAR RNA and 6 µL transfection reagent in each well) was diluted in a 500 µL transfection medium, gently mixed, and incubated for 30 minutes at room temperature. Next, the mixture was poured into the plate and incubated in a 5% CO_2 incubator at 37 C^o. After 6 hrs, 1 ml of complete RPMI medium was added to each well without discarding the transfection mixture. Twenty-four hours after transfection, cells were trypsinized in 0.25% Trypsin-EDTA (Gibco by Life Technology, USA), re-suspended in fresh media, and re-plated for cell viability and ROS, NADPH, and clonogenic cell survival assays. The harvested cells were also used for Western blotting and qRT-PCR assays.

Measurement of cell viability

The short-term effects of TIGAR silencing on tumour cell growth were assessed with a WST-1 assay kit (Boster, USA). The percentage of growth inhibition was calculated as (OD vehicle – OD treatment), where ODs were measured using a SpectraMax plus 384 microplate reader at 420 and 480 nm, respectively (Molecular Devices LLC, USA).

Clonogenic survival assay

The long-term effects of TIGAR silencing on tumour cell proliferation were analyzed employing a colony-formation assay. Twenty-four hours after siRNA transfection, 1×10^2 A549 cells were plated in triplicate in 6-well plates. The media was changed every 3 days for 12 days and then discarded; cells were washed once by PBS (Sigma-Aldrich, USA), then fixed by adding methanol: acetic acid (3:1) (Sigma-Aldrich, USA) and incubated for 5 minutes, with subsequent fixing solution removal. Staining was performed using 0.5% crystal violet (Sigma-Aldrich, USA) in methanol for 15 minutes; cells were then gently washed with distilled water and left to dry at room temperature. During microscopic observation of cells, colonies consisting of 50 cells were counted as single colonies (Chen et al., 2015).

Total cellular ROS levels

The ROS effects of TIGAR knockdown on tumour cell growth were assessed with a DCFDA assay kit (Abcam, USA). DCFDA level was measured with a fluorescence spectrometer plate reader at (Ex = 485 and Em = 535 nm utilizing end-point mode (Perkin-Elmer LS-55, USA).

Measurement of NADPH levels

The effects of TIGAR silencing on tumour cell growth was evaluated with a NADPH assay kit (Abcam, USA) to determine NADPH levels. NADPH percentage levels were calculated as (OD vehicle – OD treatment), where ODs were measured using a SpectraMax plus 384 microplate reader at (460 nm).

Protein extraction and Western blot analysis

Equal amounts of total protein extracts were analyzed in 12% (W V^{-1}) SDS–PAGE and protein bands transferred to PVDF membranes. Bovine serum albumin (5%) was used for blocking the membrane, which was then incubated with primary antibodies at 4 ^{o}C overnight with gentle shaking. The following primary antibodies were used: TIGAR (1:100); p53 (1:500); Bcl-2 (1:500); CASPASE-3 (1:500); BECLIN-1 (1:500); LC3-a (1:500); and GAPDH (1:500), all purchased from Santa Cruz Biotechnology (USA); NF-κB P65 (1:2000) and Heme Oxygenase-1 (1:2000) were purchased from Abcam (USA). The membranes were then incubated with secondary antibody that conjugated with Horseradish peroxidase for 1 hour at room temperature with gentle agitation. The membranes were washed 3 times for 5 minutes by TBS-T between each step. Thereafter, the protein bands were visualized with an enhanced chemiluminescence technique. Bands were analyzed by scanning the film and making use of Adobe Photoshop CS5 software to verify the densitometry levels (Mahmood and Yang, 2012).

RNA extraction and qRT-PCR

Total RNAs from cells were extracted using Quick-RNA™ MiniPrep kit (Zymo Research, USA). cDNA synthesis was performed from 1 µg of RNA using a cDNA Synthesis kit (Bioline, UK); a thermal cycler (Sensoquest Labcycler, Germany) machine was used. Real-time PCR amplification was performed with 4 µL of cDNA with the 2 × qPCRBIO SyGreen Mix (PCR Biosystems, UK) using a Rotor Gene PCR system (Qiagen, Germany); all steps were completed in accordance with the manufacturers' instructions. Expression mRNA was quantified; primers were purchased from Sentegen Company (Turkey) and their sequences were designed previously via the NCBI website. BCL2:Forward:GGAGGATTGTGGCCTTCTT T,Reverse:CCGTACAGTTCCACAAAGG,CAS P3:Forward:ATTGTGGAATTGATGCGTGARe verse:GCAGGCCTGAATAATGAAA,BECN1: Forward:TCACCATCCAGGAACTCACARever se:TCAGTCTTCGGCTGAGGTT,MAP1-LC3A,Forward: CAACATGAGCGAGTTGGTC, Reverse: AAGCCGTCCTCGTCTTTCTC,C12ORF5:For ward: GGCATGGAGAAACAAGATT Reverse:CATGGTCTGCTTTGTCCTCAGAPD H:Forward:CCACCCAGAAGACTGTGGAT,R everse:TTCTAGACGGCAGGTCAGGT,NFKB 1:Forward:CCTGGATGACTCTTGGGAAA,Re verse:TCAGCCAGCTGTTTCATGTC,HMOX1 :Forward:ATGACACCAAGGACCAGAGC, Reverse: GTGTAAGGACCCATCGGAGA. The CT (threshold cycle) value of TIGAR amplification was normalized to that of the GAPDH control. Gene expression was quantified relative to the housekeeping gene, GAPDH, according to the comparative ΔΔCt, Livak method (Livak and Schmittgen, 2001).

Statistical analysis

All data were expressed as means ± standard deviation. Statistical testing was performed with GraphPad Prism v5.01 software statistical package (GraphPad). A paired t-test was employed for comparing the two groups. Significant differences at $p < 0.05$, 0.01 and 0.001 are indicated by *, **, and ***, respectively.

RESULTS AND DISSCUSSION

TIGAR knockdown affects A549 growth rate and colony formation

In accordance with the scope of this study, we sought to explore the biological consequences of specific silencing of TIGAR in the A549 cell line. We evaluated both short- and long-term cell viability via a clonogenic assay and cell proliferation assay, respectively. We investigated TIGAR inhibition by siRNA and the results showed there was both inhibition of cell growth and colony formation in A549 cells. A WST-I assay (Fig 1-A) indicated that treating A549 cells using siTIGAR inhibited cell viability. Reduced A549 cell viability was strongly related to silencing of TIGAR following siTIGAR treatment. After TIGAR was down-regulated by RNAi for 24 hours, cell viability decreased noticeably compared to an untreated control (Fig 1-A). A549 TIGAR-silenced cells also showed there to be a clear reduction of colony formation when compared to control cells (Fig 1-B).

To investigate how siRNA TIGAR could affect cell growth and cell survival, we designed several experiments specifically for such purposes. For this reason, A549 cell growth was studied 24 hours post-transfection by cell viability assay via the WST-1 kit. Cells transfected with TIGAR siRNA exhibited a decrease in growth significantly less than the control cells. Consequently, colony formation was markedly lower than in the control condition.

To determine whether the decrease in cell numbers following TIGAR knockdown took place because of cell death, we assessed several mechanisms. First, we analyzed protein expression and mRNA levels of CASPASE-3, an apoptotic marker that is significantly elevated after silencing of TIGAR 24 hours post-transfection (Fig 1-E and Fig 2-F). We also examined type-II programmed cell death, autophagy markers, because in response to anticancer therapies, different types of cancer cells undergo autophagy, though whether autophagy in cancer protects or kills cells remains a subject of debate (Zhuang et al., 2009). Similar results of up-regulation were obtained for autophagy markers (light chain 3) LC3 I, LC3 II, and BECLIN-1 (Fig 2).

TIGAR-specific siRNA up-regulates apoptosis and autophagy markers

Previous studies have reported siRNA TIGAR down-regulated TIGAR expression in glioblastoma cells, hepatocellular carcinoma, and prostate cancer (Wanka et al., 2012; Xie et al., 2014; Yu et al., 2015; Tai et al., 2016; Huang et al.; 2017). Also, they stated that both types of programmed cell death-autophagy and apoptosis-could occur in mammalian cells, and the induction of autophagy has been shown to enhance the apoptotic response (Zhao et al., 2017a). To establish which cell death mechanism in A549 cells existed in TIGAR-silenced cells, we first observed silencing of TIGAR expression by siRNA, which can activate cell death signalling in A549 cells. Therefore, A549 cells were transfected with siRNAs targeting TIGAR. Twenty-four hours after transfection, the cells were used in both Western blots and qRT-PCR assays to verify the levels of apoptosis, autophagy. The results indicated that cell growth was inhibited, with evidence of apoptotic and autophagic cell death after TIGAR silencing in A549 cells. According to previous studies, programmed cell death I and II may also occur when TIGAR silencing is combined with certain drugs, including adriamycin, rapamycin, epirubicin, cisplatin, or an external factor, such as hypoxia or radiotherapy (Pena-Rico et al., 2011; Wanka et al., 2012; Xie et al., 2014; Yu et al., 2015;

Huang et al., 2017). The results suggested that protein expression and mRNA levels of apoptotic marker, CASPASE-3, had significantly increased after silencing of TIGAR (Fig 1-E and Fig 1-F). Additionally, up-regulation of protein and mRNA levels was evident for both autophagy markers, LC3 I and II, and in BECLIN-1 (Fig 2).

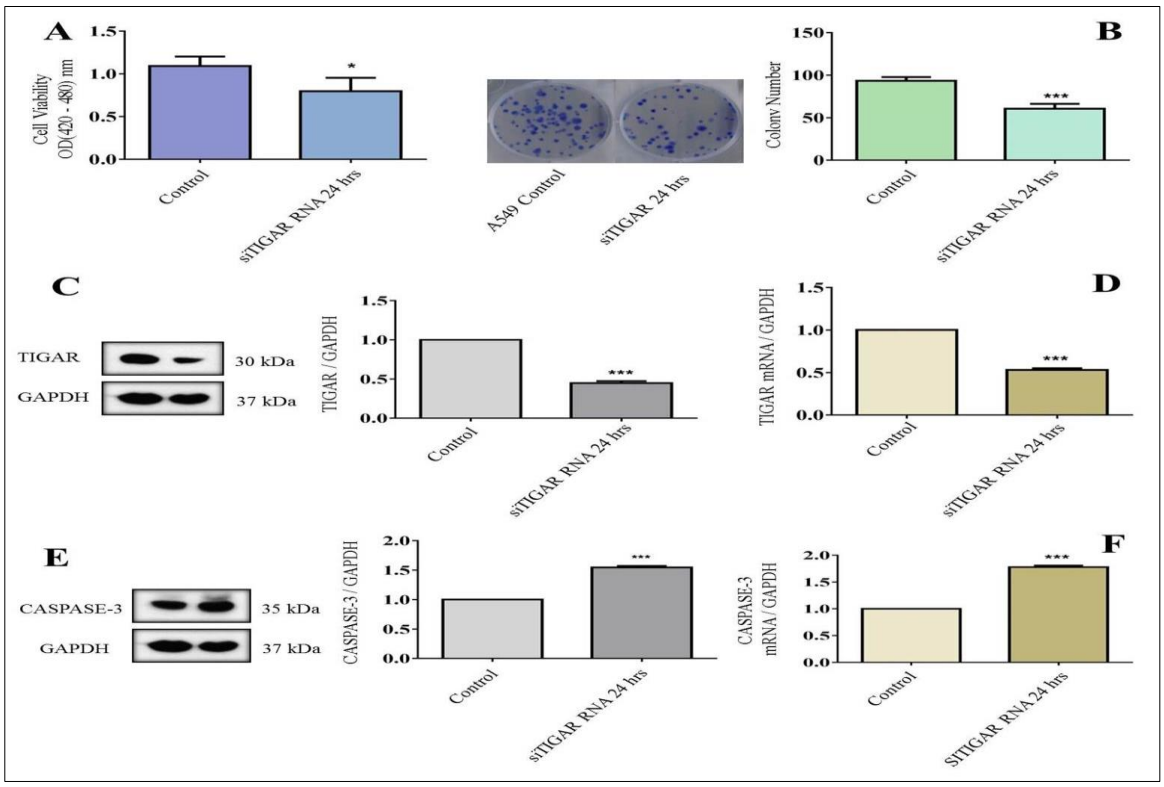

Figure 1. siRNA TIGAR knockdown inhibited viability and induced apoptosis of A549 cells. (A) Short-term effects of cell growth were assayed by a WST-1 assay. (B) Long-term effects of cell growth were assayed by a colony formation assay. (C) and (D) Expression of TIGAR protein and mRNA levels. (E) and (F) Expression of CASPASE-3 protein and mRNA levels. Data were expressed as mean ± SD with three independent experiments. (* $p< 0.05$ vs cont., ** $p<0.01$ vs cont. and *** $p<0.001$ vs cont.).

The studies indicate that autophagy and apoptosis play dual roles in cell death (Ye et al., 2013), demonstrating that differentiation malignancy of NSCLC is closely correlated with TIGAR expression. By inducing the PPP, TIGAR can limit fructose-2, 6-bisphosphatase concentrations. Moreover, recent studies have also illustrated TIGAR's relationship to autophagy and apoptosis, showing that TIGAR's silencing ability is strongly connected to up-regulation of ROS, resulting in higher intracellular NADPH levels (Xie et al., 2014). Our findings provide evidence that TIGAR is essential for programmed cell death via apoptosis and autophagy.

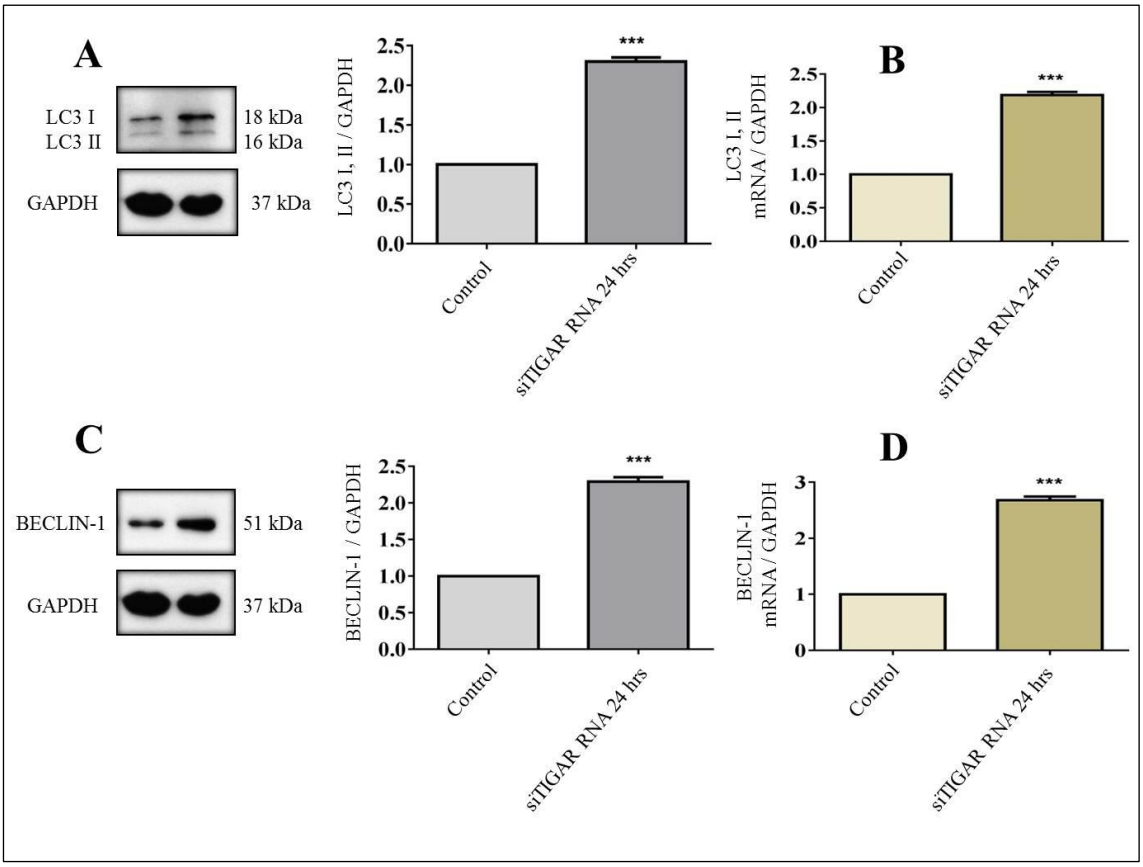

Figure 2. siRNA TIGAR knockdown enhances autophagy by repressing TIGAR expression. (A) and (B) Expression of LC3 I and II proteins and mRNA levels. (C) and (D) Expression of BECLIN-1 protein and mRNA levels. Data were expressed as mean ± SD with three independent experiments. (* $p< 0.05$ vs cont., ** $p<0.01$ vs cont. and *** $p<0.001$ vs cont.).

TIGAR knockdown induces an increase ROS and oxidative stress markers

To determine whether silencing TIGAR was associated with increased ROS levels, we measured ROS levels in cells transfected with TIGAR siRNA. The results indicated that ROS levels were slightly increased in A549 cells after TIGAR knockdown (Fig 3-A). Furthermore, NADPH levels were assessed to establish whether decreasing viability and increasing cell death induced by silencing TIGAR were related to NADPH levels, so we verified NADPH levels in the cells transfected with TIGAR siRNA. The results showed that NADPH levels diminished slightly in A549 cells after TIGAR knockdown (Fig 3-B). Down-regulation of NADPH and up-regulation of ROS levels coincided with a decrease in TIGAR protein and increased cell death in other associated proteins as a consequence of TIGAR knockdown (Figs 1 and 2).

A number of studies of cancer cells have shown that TIGAR silencing is related to decreased levels of NADPH (Lui et al., 2010; Yin et al., 2012) and lower levels of GSH (Ye et al., 2013; Wong et al., 2015); this is consequently followed by an increase in ROS (Qian et al., 2016). Pen˜a-Rico and colleagues demonstrated that TIGAR knockdown resulted in radio-sensitization of glioma cells with elevated levels of Fru-2, 6-BP, lactate, and ROS, as well as reduced GSH levels (Pena-Rico et al., 2011). The PPP, also known as the phosphor-gluconate pathway, owing to its role in supplying cancer cells with ribo-nucleotides and

NADPH for intracellular anabolic processes, plays a pivotal role in apoptosis, tumour proliferation, resistance to immune radiation and chemotherapy, and angiogenesis (Patra and Hay, 2014). High levels of PPP flux are known to lead to more proliferative, invasive, and drug-resistant phenotypes in cancer cells (Riganti et al., 2012) and may be critical for cancer treatment if the PPP is inhibited. In addition, to understand the relationship of oxidative stress markers to cell death types, such as autophagy and apoptosis, we indicated that the expression of them were up-regulated 24 hours post-siTIGAR transfection (Fig 3-C, D, E and F) when the effect of siTIGAR on the expression of both NF-κB and HO-1 were measured by Western blotting and qRT-PCR analysis. Furthermore, increased ROS levels are also seen with enhanced levels of the oxidative stress markers, NF-κB and HO-1 (Fig 3), which may be responsible for induction of both autophagy and apoptosis.

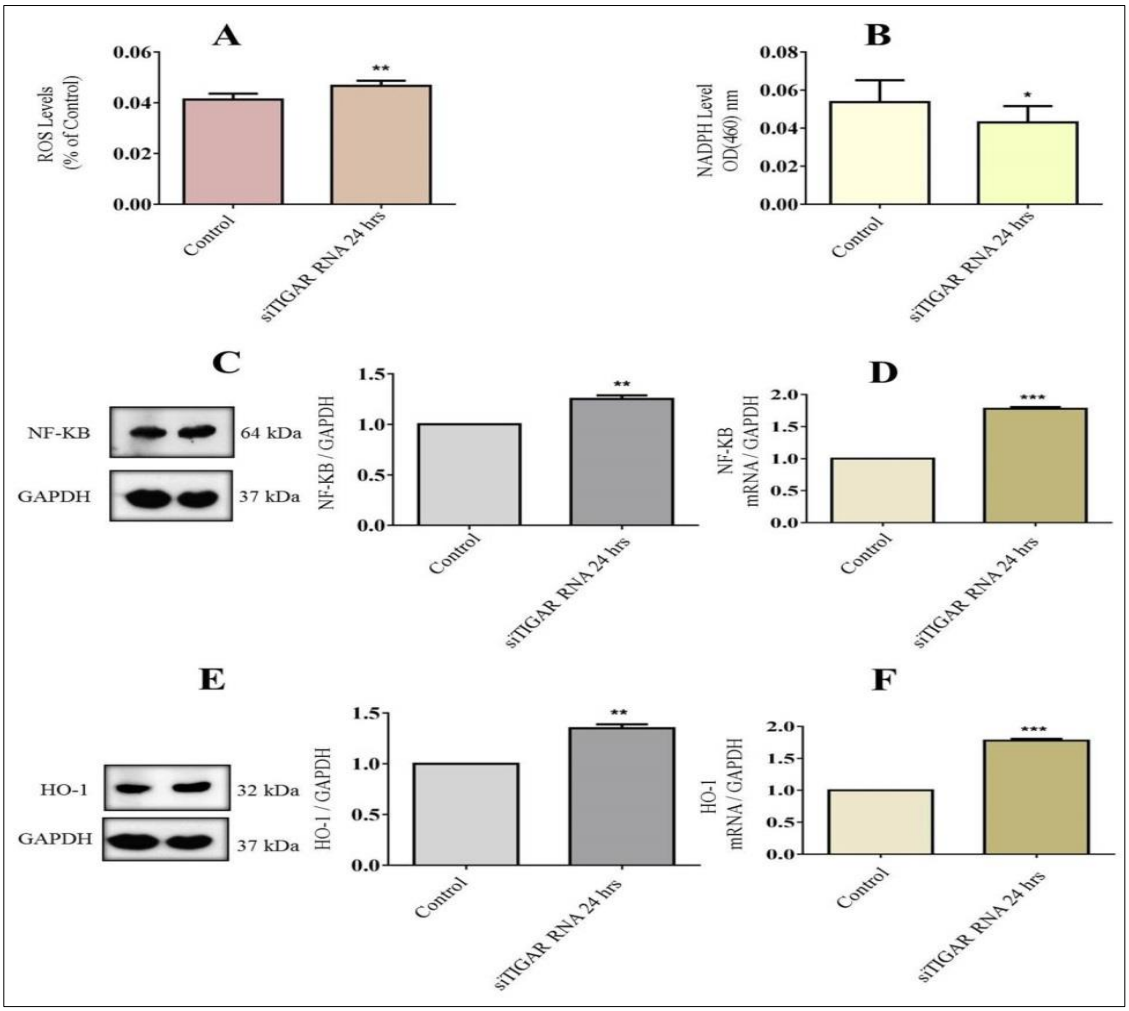

Figure 3. siRNA TIGAR knockdown enhances the expression of NF-κB and HO-1 through increasing ROS and decreasing NADPH levels. (A) ROS levels were detected by DCFDA assay. (B) NADPH levels were detected. (C) and (D) NF-κB protein and mRNA expression. (E) and (F) HO-1 protein and mRNA expression. Data were expressed as mean ± SD with three independent experiments. (* $p< 0.05$ vs cont., ** $p<0.01$ vs cont. and *** $p<0.001$ vs cont.).

CONCLUSION

Targeting TIGAR with siRNA caused specific and efficient inhibition of endogenous TIGAR expression in A549 lung cancer cells. TIGAR silencing inhibits cell viability and colony formation and induces the accumulation of intracellular ROS while reducing NADPH levels that consequently cause DNA damage. Impairment induces an elevation of the oxidative stress makers, as well, so consequently cell death was observed. These data suggest that TIGAR-regulated ROS and induced cell death via ROS may be used as potential strategies in cancer therapy. This new strategy has the potential to be molecularly targeted through opening a new avenue in gene silencing therapy for lung cancer cells.

ACKNOWLEDGEMENTS

This work was supported by TUBITAK-SBAG (Project no: 215S192).

REFERENCES

Bansal S, Biswas G, Avadhani NG, 2014. Mitochondria-targeted heme oxygenase-1 induces oxidative stress and mitochondrial dysfunction in macrophages, kidney fibroblasts and in chronic alcohol hepatotoxicity. Redox biology, 2: 273-283.

Bensaad K, Cheung EC, Vousden KH, 2009. Modulation of intracellular ROS levels by TIGAR controls autophagy. The EMBO journal, 28: 3015-3026.

Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH, 2006. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell, 126: 107-120.

Bindu S, Pal C, Dey S, Goyal M, Alam A, Iqbal MS, Dutta S, Sarkar S, Kumar R, Maity P , et al., 2011. Translocation of heme oxygenase-1 to mitochondria is a novel cytoprotective mechanism against non-steroidal anti-inflammatory drug-induced mitochondrial oxidative stress, apoptosis, and gastric mucosal injury. The Journal of biological chemistry, 286: 39387-39402.

Chen S, Li P, Li J, Wang Y, Du Y, Chen X, Zang W, Wang H, Chu H, Zhao G , et al., 2015. MiR-144 inhibits proliferation and induces apoptosis and autophagy in lung cancer cells by targeting TIGAR. Cellular physiology and biochemistry, 35: 997-1007.

Cheung EC, Lee P, Ceteci F, Nixon C, Blyth K, Sansom OJ, Vousden KH, 2016. Opposing effects of TIGAR- and RAC1-derived ROS on Wnt-driven proliferation in the mouse intestine. Genes & development, 30: 52-63.

Gazdar AF, Girard L, Lockwood WW, Lam WL, Minna JD, 2010. Lung cancer cell lines as tools for biomedical discovery and research. Journal of the National Cancer Institute, 102: 1310-1321.

Gloire G, Legrand-Poels S, Piette J, 2006. NF-kappaB activation by reactive oxygen species: fifteen years later. Biochemical pharmacology, 72: 1493-1505.

Han Z, Varadharaj S, Giedt RJ, Zweier JL, Szeto HH, Alevriadou BR, 2009. Mitochondria-derived reactive oxygen species mediate heme oxygenase-1 expression in sheared endothelial cells. The Journal of pharmacology and experimental therapeutics, 329: 94-101.

Huang S, Yang Z, Ma Y, Yang Y, Wang S, 2017. miR-101 Enhances Cisplatin-Induced DNA Damage Through Decreasing Nicotinamide Adenine Dinucleotide Phosphate Levels by Directly Repressing Tp53-Induced Glycolysis and Apoptosis Regulator Expression in Prostate Cancer Cells. DNA and cell biology, 36: 303-310.

Huang T, Zhang P, Li W, Zhao T, Zhang Z, Chen S, Yang Y, Feng Y, Li F, Shirley Liu X , et al., 2017. G9A promotes tumor cell growth and invasion by silencing CASP1 in non-small-cell lung cancer cells. Cell death & disease, 8: e2726.

Lee P, Vousden KH, Cheung EC, 2014. TIGAR, TIGAR, burning bright. Cancer & metabolism, 2: 1.

Leung CC, Porcel JM, Takahashi K, Restrepo MI, Lee P, Wainwright C, 2014. Year in review 2013: Lung cancer, respiratory infections, tuberculosis, cystic fibrosis, pleural diseases, bronchoscopic intervention and imaging. Respirology, 19: 448-460.

Li L, Tan J, Miao Y, Lei P, Zhang Q, 2015. ROS and Autophagy: Interactions and Molecular Regulatory Mechanisms. Cellular and molecular neurobiology, 35: 615-621.

Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods, 25: 402-408.

Lui VW, Lau CP, Cheung CS, Ho K, Ng MH, Cheng SH, Hong B, Tsao SW, Tsang CM, Lei KI , et al., 2010. An RNA-directed nucleoside anti-metabolite, 1-(3-C-ethynyl-beta-d-ribo-pentofuranosyl) cytosine (ECyd), elicits antitumor effect via TP53-induced Glycolysis and Apoptosis Regulator (TIGAR) downregulation. Biochemical pharmacology, 79: 1772-1780.

Lui VW, Wong EY, Ho K, Ng PK, Lau CP, Tsui SK, Tsang CM, Tsao SW, Cheng SH, Ng MH , et al., 2011. Inhibition of c-Met downregulates TIGAR expression and reduces NADPH production leading to cell death. Oncogene, 30: 1127-1134.

Mahmood T, Yang PC, 2012. Western blot: technique, theory, and trouble shooting. North American journal of medical sciences, 4: 429-434.

Morgan MJ, Liu ZG, 2011. Crosstalk of reactive oxygen species and NF-kappaB signaling. Cell research 21: 103-115.

Patra KC, Hay N, 2014. The pentose phosphate pathway and cancer. Trends in biochemical sciences 39: 347-354.

Pena-Rico MA, Calvo-Vidal MN, Villalonga-Planells R, Martinez-Soler F, Gimenez-Bonafe P, Navarro-Sabate A, Tortosa A, Bartrons R, Manzano A, 2011. TP53 induced glycolysis and apoptosis regulator (TIGAR) knockdown results in radiosensitization of glioma cells. Radiotherapy and oncology, 101: 132-139.

Qian S, Li J, Hong M, Zhu Y, Zhao H, Xie Y, Huang J, Lian Y, Li Y, Wang S , et al., 2016. TIGAR cooperated with glycolysis to inhibit the apoptosis of leukemia cells and associated with poor prognosis in patients with cytogenetically normal acute myeloid leukemia. Journal of hematology & oncology, 9: 128.

Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K, 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. BioMed research international, 2014: 1-19.

Riganti C, Gazzano E, Polimeni M, Aldieri E, Ghigo D, 2012. The pentose phosphate pathway: an antioxidant defense and a crossroad in tumor cell fate. Free radical biology & medicine, 53: 421-436.

Scherz-Shouval R, Elazar Z, 2007. ROS, mitochondria and the regulation of autophagy. Trends in cell biology, 17: 422-427.

Tai G, Gu P, Zhang H, He X, Du J, Huang J, Yu J, Cai J, Liu F, 2016. TP53 induced glycolysis and apoptosis regulator knockdown radiosensitizes glioma cells through oxidative stress-induced excessive autophagy. International Journal of Clinical and Experimental Pathology, 9: 7.

Wang S, Yu Q, Zhang R, Liu B, 2011. Core signaling pathways of survival/death in autophagy-related cancer networks. International Journal of Biochemistry and Cell Biology, 43: 1263-1266.

Wang Y, Huang X, Cang H, Gao F, Yamamoto T, Osaki T, Yi J, 2007. The endogenous reactive oxygen species promote NF-kappaB activation by targeting on activation of NF-kappaB-inducing kinase in oral squamous carcinoma cells. Free radical research, 41: 963-971.

Wanka C, Steinbach JP, Rieger J, 2012. Tp53-induced glycolysis and apoptosis regulator (TIGAR) protects glioma cells from starvation-induced cell death by up-regulating respiration and improving cellular redox homeostasis. The Journal of biological chemistry, 287: 33436-33446.

Wong EY, Wong SC, Chan CM, Lam EK, Ho LY, Lau CP, Au TC, Chan AK, Tsang CM, Tsao SW , et al., 2015. TP53-induced glycolysis and apoptosis regulator promotes proliferation and invasiveness of nasopharyngeal carcinoma cells. Oncology letters, 9: 569-574.

Xie JM, Li B, Yu HP, Gao QG, Li W, Wu HR, Qin ZH, 2014. TIGAR has a dual role in cancer cell survival through regulating apoptosis and autophagy. Cancer research, 74: 5127-5138.

Ye L, Zhao X, Lu J, Qian G, Zheng JC, Ge S, 2013. Knockdown of TIGAR by RNA interference induces apoptosis and autophagy in HepG2 hepatocellular carcinoma cells. Biochemical and biophysical research communications, 437: 300-306.

Yin L, Kosugi M, Kufe D, 2012. Inhibition of the MUC1-C oncoprotein induces multiple myeloma cell death by down-regulating TIGAR expression and depleting NADPH. Blood, 119: 810-816.

Yu HP, Xie JM, Li B, Sun YH, Gao QG, Ding ZH, Wu HR, Qin ZH, 2015. TIGAR regulates DNA damage and repair through pentosephosphate pathway and Cdk5-ATM pathway. Scientific reports, 5: 9853.

Zhao D, Han W, Liu X, Cui D, Chen Y, 2017. MicroRNA-128 promotes apoptosis in lung cancer by directly targeting NIMA-related kinase 2. Thoracic cancer, 8: 304-311.

Zhuang W, Qin Z, Liang Z, 2009. The role of autophagy in sensitizing malignant glioma cells to radiation therapy. Acta biochimica et biophysica Sinica, 41: 341-351.