Biochemical and Biophysical Research Communications 443 (2014) 598–603

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

GSK3β negatively regulates HIF1α mRNA stability via nucleolin in the MG63 osteosarcoma cell line

Dong-dong Cheng [a,1], Hai-guang Zhao [b,1], Yun-song Yang [c], Tu Hu [a], Qing-cheng Yang [a,*]

[a] Department of Orthopedics, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China
[b] Department of Vascular Surgery, Shanghai Jiao Tong University Affiliated Ninth People's Hospital, Shanghai 200011, China
[c] Huazhong University of Science and Technology, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 28 November 2013
Available online 11 December 2013

Keywords:
GSK3β
HIF1α
mRNA
Nucleolin
Osteosarcoma

ABSTRACT

Hypoxia-inducible factor 1α (HIF1α) is a transcription factor involved in the growth, invasion and metastasis of malignant tumors. Glycogen synthase kinase 3 beta (GSK3β) is a protein kinase involved in a variety of signaling pathways, such as the Wnt and NF-κB pathways; this kinase can affect tumor progress through the regulation of transcription factor expression and apoptosis. Recent studies showed that GSK3β was involved in the expression of HIF1α. However, the effect of GSK3β on HIF1α expression in osteosarcoma cells remains unknown. To understand the relationship between GSK3β and HIF1α comprehensively, small RNA interference techniques, Western blot analyses, quantitative real-time PCR analyses and luciferase assays were used in our study. Experimental data revealed that inhibition of GSK3β could increase HIF1α protein levels and expression of its target genes by increasing the stability of the HIF1α mRNA, not by affecting the HIF1α protein stability, and that this process could be mediated by nucleolin.

1. Introduction

Normally mammalian cells need sufficient levels of oxygen to survive. In fact, hypoxia is a common feature of many diseases, e.g., a hypoxic environment exists in almost all solid tumors. Under hypoxic conditions, cells must compensate for the lack of oxygen. HIF1α is the main regulator of tumorigenesis and tumor angiogenesis under hypoxic conditions and regulates the expression of more than 100 genes, including oxygen transport, glucose metabolism, cell proliferation and apoptosis [1–3]. Under hypoxic conditions, HIF can promote the expression of its target genes, including VEGF, EPO and GLUT1 [4]. HIF-1 is composed of two subunits, namely HIF1α and ARNT [5]. HIF1α is encoded by the HIF1A gene and activated by hypoxia. Its activation is mediated by phosphorylation while its degradation occurs through a proteasome-mediated pathway [6]. The stability of HIF1α is primarily regulated through the hydroxylation of two proline residues (P402 and P564) in the oxygen-dependent degradation (ODD) domain. After hydroxylation, HIF1α binds to the tumor suppressor protein von Hippel-Lindau (VHL), which is an E3 ubiquitin ligase that initiates the ubiquitination of HIF1α and promotes its proteasomal degradation [7,8]. Recent studies have found that HIF1α is degraded through a VHL independent process [9]. Nonetheless, the mechanism of HIF1α degradation in osteosarcoma remains unknown.

GSK3 is a multifunctional serine/threonine kinase first discovered in rabbit skeletal muscle 30 years ago, which inactivates glycogen synthase by phosphorylation [10,11]. GSK3 is associated with the development of many human diseases, such as diabetes, inflammation, diseases of the nervous system and various malignant tumors [12,13]. So far, two members of the mammalian GSK3 family, which belong to the CMGC protein kinase family, have been identified, namely GSK3α and GSK3β [11]. They are widely expressed in multiple tissues with highly conserved structures [14]. The activity of GSK3β is regulated by phosphorylation of specific amino acid residues (Tyr 216/serine 9) [15]. Phosphorylation of serine 9 is the main cause of various pathological changes and it inactivates GSK3β in a variety of tumors [16]. Many upstream protein kinases, such as PKA, Akt/PKB, PKC and MAPKAP, can phosphorylate GSK3β, and the functions of these upstream signaling molecules have been repeatedly reported [17,18]. Recent studies have shown that GSK3β affects tumor development and

Abbreviations: HIF1α, hypoxia-inducible factor 1α; GSK3β, glycogen synthase kinase 3 beta; VEGF, vascular endothelial growth factor; EPO, erythropoietin; GLUT1, glucose transporter 1; ARNT, aryl hydrocarbon receptor nuclear translocator; ODD, oxygen-dependent degradation; VHL, von Hippel-Lindau; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Chx, cycloheximide; siRNA, small interfering RNA.

* Corresponding author. Address: Department of Orthopedics, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, No.600, Yishan Road, Shanghai 200233, China. Fax: +86 21 64701361.
E-mail address: tjyqc@163.com (Q.-c. Yang).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.020

progression by regulating the expression of transcription factors, the cell cycle and apoptosis [16]. It is interesting to note that the role of GSK3β in tumors is associated with the tumor cell type, which means it promotes growth in some tumors, while doing the opposite in others [16].

It has been reported that GSK3β can regulate HIF1α; however, the underlying mechanisms still remain incompletely understood. Daniela Flügel reported that GSK3β regulated HIF1α via three key amino residues in the ODD domain in the HepG2 cell line and mutation analysis revealed that GSK3β degraded HIF1α in a VHL independent way [19]. In their following study, they found that GSK3β regulated cell growth, migration and angiogenesis via Fbw7 and USP28-dependent degradation of HIF1α [9].

As is reported in other tumors, our research found that in osteosarcoma inhibition of GSK3β promoted the expression of HIF1α and its target genes, which was achieved by regulation of the HIF1α mRNA, rather than HIF1α protein stability. Furthermore, this process could be mediated through the regulation of nucleolin, which has not been previously reported in the literature.

2. Materials and methods

2.1. Cell culture

The MG63 cell line was purchased from the ATCC. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Biowest, South America Origin), 100 U/mL penicillin (Sigma–Aldrich) and 100 μg/mL streptomycin (Sigma–Aldrich) at 37 °C in 5% CO_2. The cells were regularly certified as free of mycoplasma contamination.

2.2. Antibodies and reagents

Antibodies against GSK3β, GSK3α, β-catenin and the HA tag were purchased from Cell Signaling Technology, the Flag tag antibody was purchased from Sigma, the HIF1α antibody was purchased from BD Biosciences and the tubulin antibody was purchased from Santa Cruz Biotechnology. The GSK3β specific inhibitor TDZD-8, proteasome inhibitor MG132, cycloheximide and actinomycin D were purchased from Sigma.

2.3. siRNA and transfection

The siRNAs against GSK3β and nucleolin, negative control siRNA and DharmaFECT transfection reagent were purchased from Thermo Scientific. 40 nM siRNA was transfected into cells following the manufacturer's protocol.

2.4. Plasmids and transfection

Flag-HIF1αP402A, P564G (Flag-HIF1α2pm) has been described previously [20] and was kindly provided by Dr. Jaime Caro (Thomas Jefferson University). Flag-HIF1αP402A, P564G, S551A, T555V, S589A (Flag-HIF1α2pmSTSm) was generated using Flag-HIF1α2pm as a template with a site directed mutagenesis kit (Stratagene). pcDNA3-HA-GSK3β was produced by PCR using MG63 cell cDNA as template. pGL2-HIF1A−572 to +284 was produced by PCR using MG63 cell genomic DNA as template. The plasmids were validated by DNA sequencing. Transfections were performed using Lipofectamine 2000 (Invitrogen) based on the manufacturer's instructions.

2.5. Western blot analysis

Equal amounts of protein extracted from the harvested cells were resolved by 5–8% SDS–PAGE. After electrophoresis, the proteins were electroblotted onto NC/PVDF membranes (Millipore, USA). The blots were blocked using 5% (w/v) nonfat dry milk in PBS overnight at 4 °C prior to immunoprobing with antibodies diluted in PBS with 5% (w/v) milk for 2 h at room temperature. The membranes were incubated overnight at 4 °C with the indicated antibodies and then incubated with secondary antibodies for 2 h. The subsequent visualization was performed using SuperSignal West Femto Maximun Sensitivity Substrate (Thermo, Japan) with tubulin levels used as the loading control. Three independent assays were conducted.

2.6. RNA isolation and quantitative real-time PCR

Total RNA was extracted from the cultured cells using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol and quantified with the Nanodrop 2000 (Thermo, Japan). First-strand cDNA synthesis and amplification were performed using reverse transcription reagents (Takara, China) following the manufacturer's instructions. The cDNA templates were combined with SYBR Green premix with RoxII (Takara, China) to perform quantitative-PCR reactions. GADPH was used as the endogenous control for quantifying mRNA levels. Three independent assays were performed. Details of the primer sequences used are presented in Supplementary Table 1.

2.7. Luciferase assay

The luciferase assay and β-gal assay kits were purchased from Thermo Scientific. pGL2-HIF1A−572 to +284 and the β-gal plasmid were cotransfected into MG63 cells and β-gal levels were measured as a transfection efficiency control.

2.8. Statistics

The results are presented as the mean ± SEM. Comparisons of quantitative data were analyzed by Student's *t*-test ($p < 0.05$ was considered to be significant). $^{*}P < 0.05$, $^{**}P < 0.01$.

3. Results

3.1. Inhibition of GSK3β enhanced HIF1α protein expression and expression of its target genes in osteosarcoma cells

To test the effect of GSK3β on HIF1α expression in osteosarcoma cells, we knocked down GSK3β expression in MG63 cells, as shown in Fig. 1A, which remarkably enhanced HIF1α expression. Similar results were obtained in cells treated with a specific GSK3β inhibitor (Fig. 1B). Levels of β-catenin, a putative GSK3β target gene, were increased by GSK3β inhibition. The mRNA expression of the HIF target genes VEGF and Glut1 were evaluated in these cells. Fig. 1C shows that the knock down of GSK3β significantly increased the mRNA expression of VEGF (a) and Glut1 (b), suggesting that inhibition of GSK3β enhanced HIF1α protein expression and the expression of its target genes in osteosarcoma cells.

3.2. GSK3β did not affect HIF1α protein stability in osteosarcoma cells

Previous studies have shown that GSK3β phosphorylates three amino acid residues in the ODD domain of HIF1α, mediating a VHL-independent proteasomal degradation of HIF1α [9,19]. To test this in osteosarcoma cells, we knocked down GSK3β expression in MG63 cells, exposed the cells to extreme hypoxia plus the hypoxia mimic deferoxamine to completely rule out the effect of oxygen on HIF1α stability, used cycloheximide (Chx) to stop protein synthesis

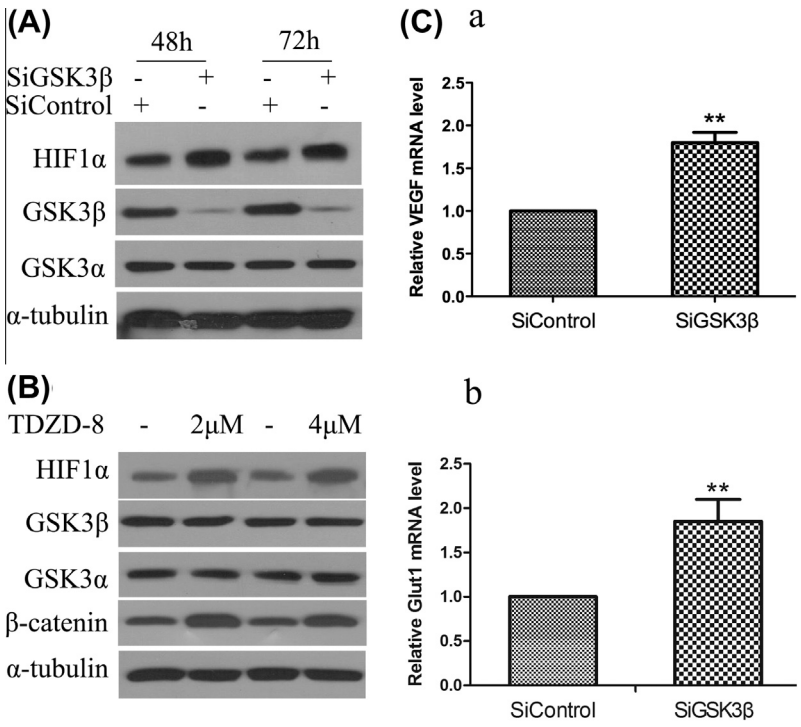

Fig. 1. Inhibition of GSK3 activity in osteosarcoma cells induces expression of HIF1α and its target genes. (A) The cells were infected with SiGSK3β or SiControl for the indicated times. (B) The cells were treated with the GSK3β inhibitor TDZD-8 at the indicated concentrations. (A, B) The cells were lysed and equal amounts of protein were separated and submitted to Western blot analysis using the indicated antibodies. (C) Total RNA was isolated from SiControl- or SiGSK3β-treated cells. VEGF and Glut1 mRNA expression was analyzed and quantified by real-time PCR as described in the Section 2. The relative levels of VEGF and Glut1 expression in SiControl-treated cells were set at 1. $^{**}P < 0.01$.

for the indicated time periods, harvested the protein and subjected it to Western blot analysis to determine the stability of HIF1α. As shown in Fig. 2A, although knocking down GSK3β increased HIF1α expression at each time point, the decay rates seemed very similar, suggesting that GSK3β most likely does not affect HIF1α protein stability in osteosarcoma cells. To further confirm the above observation, we mutated the putative GSK3β phosphorylation sites in ODD domain by site directed mutagenesis and transfected this and its parental plasmid into MG63 cells. We found that these mutations did not affect HIF1α protein stability (Fig. 2B). Likewise, knocking down or overexpression of GSK3β in MG63 cells did not affect HIF1α2pm stability (Fig. 2C and D). Altogether, our data suggest that GSK3β does not affect HIF1α protein stability in osteosarcoma cells.

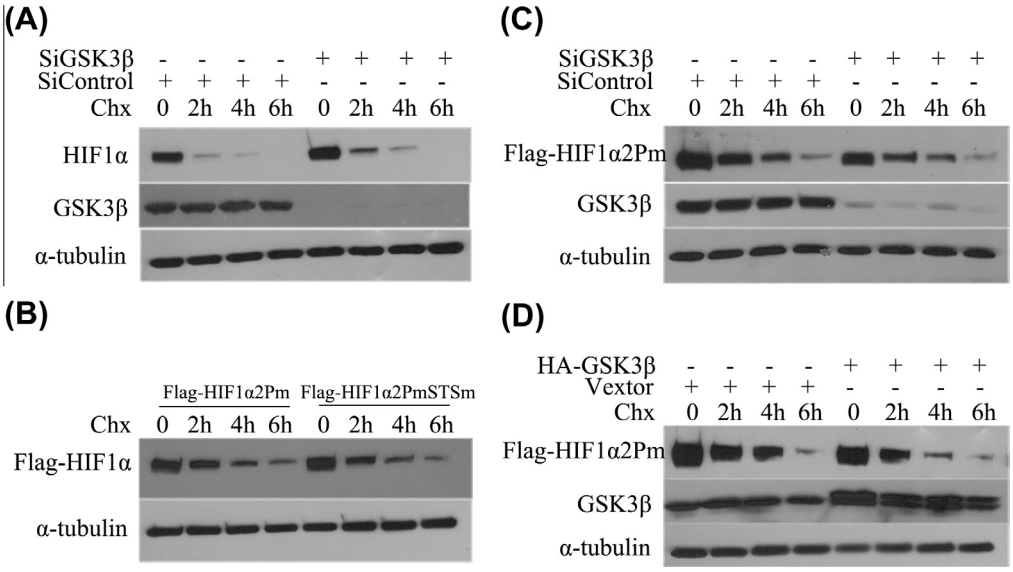

Fig. 2. GSK3β did not affect HIF1α protein stability in osteosarcoma cells. (A) The cells were infected with SiGSK3β or SiControl for the indicated times. (B) Flag-HIF1α2Pm plasmid or Flag-HIF1α2PmSTSm plasmid was transfected into MG-63 cells. (C) The cells were treated with the SiGSK3β or SiControl at the indicated concentrations. (D) The cells were treated with the HA-GSK3β or vector at the indicated concentrations. (A–D) Cycloheximide (Chx) was used to stop protein synthesis for the indicated times. The cells were lysed and equal amounts of protein were separated and submitted to Western blot analysis using the indicated antibodies.

3.3. Inhibition of GSK3β upregulated HIF1α mRNA expression and enhanced its stability in osteosarcoma cells

The above data suggest that GSK3β could regulate HIF1α expression through its effect on HIF1α mRNA expression in osteosarcoma cells. To test this hypothesis, we knocked down GSK3β expression in MG63 cells and performed quantitative RT-PCR to determine HIF1α mRNA expression. As shown in Fig. 3A, knocking down GSK3β significantly upregulated HIF1α mRNA expression. To further confirm this result, we treated MG63 cells with the MG132 proteasome inhibitor to block HIF1α protein degradation at the indicated time periods to evaluate whether knocking down GSK3β affected the accumulation of the HIF1α protein. As expected, knocking down GSK3β significantly enhanced HIF1α protein accumulation (Fig. 3B). This result also ruled out the possibility that GSK3β could affect the stability of the HIF1α protein in MG63 cells, because if that were the case, the accumulation of the HIF1α protein would be similar. Similar observations were obtained when the MG63 cells were treated with a specific GSK3β inhibitor TDZD-8 (Fig. 3C). Collectively, these data indicate that inhibition of GSK3β upregulated HIF1α mRNA expression in osteosarcoma cells. Next, we tried to determine the underlying mechanisms behind the GSK3β regulation of HIF1α mRNA expression. We cloned the HIF1A promoter region −572 to +284 into the pGL2 basic vector [21] and transfected the resulting plasmid into MG63 cells to test if GSK3β inhibition affected HIF1A promoter activity. Fig. 3D shows that knocking down GSK3β did not affect HIF1A promoter activity. Similar results were obtained when the cells were treated with the specific GSK3β inhibitor TDZD-8 (data not shown). These data suggest that GSK3β does not regulate HIF1α transcription. Another possibility is that GSK3β could affect HIF1α mRNA stability. To test this hypothesis, we knocked down GSK3β expression in MG63 cells and then blocked mRNA synthesis by treating the cells with actinomycin D. The relative HIF1α mRNA levels were measured by quantitative RT-PCR, as shown in Fig. 3E. After treatment with actinomycin D for 8 h, we found that the relative HIF1α mRNA levels in the GSK3β-knocked down cells were higher than in control cells, suggesting that knocking down GSK3β increased HIF1α mRNA stability in the MG63 cells.

3.4. Inhibition of GSK3β upregulated nucleolin mRNA expression in osteosarcoma cells

A previous study has shown that nucleolin physically binds to HIF1α mRNA and maintains its stability [22]. To test the role of nucleolin in GSK3β mediated HIF1α mRNA stability, we first determined whether GSK3β affected nucleolin expression. As shown in Fig. 4A and B, knocking down GSK3β in MG63 cells significantly increased nucleolin mRNA and protein levels. Next, we examined if nucleolin contributed to the stability of HIF1α mRNA in MG63 cells by knocking down expression of nucleolin, which significantly decreased HIF1α mRNA and protein expression (Fig. 4C and D).

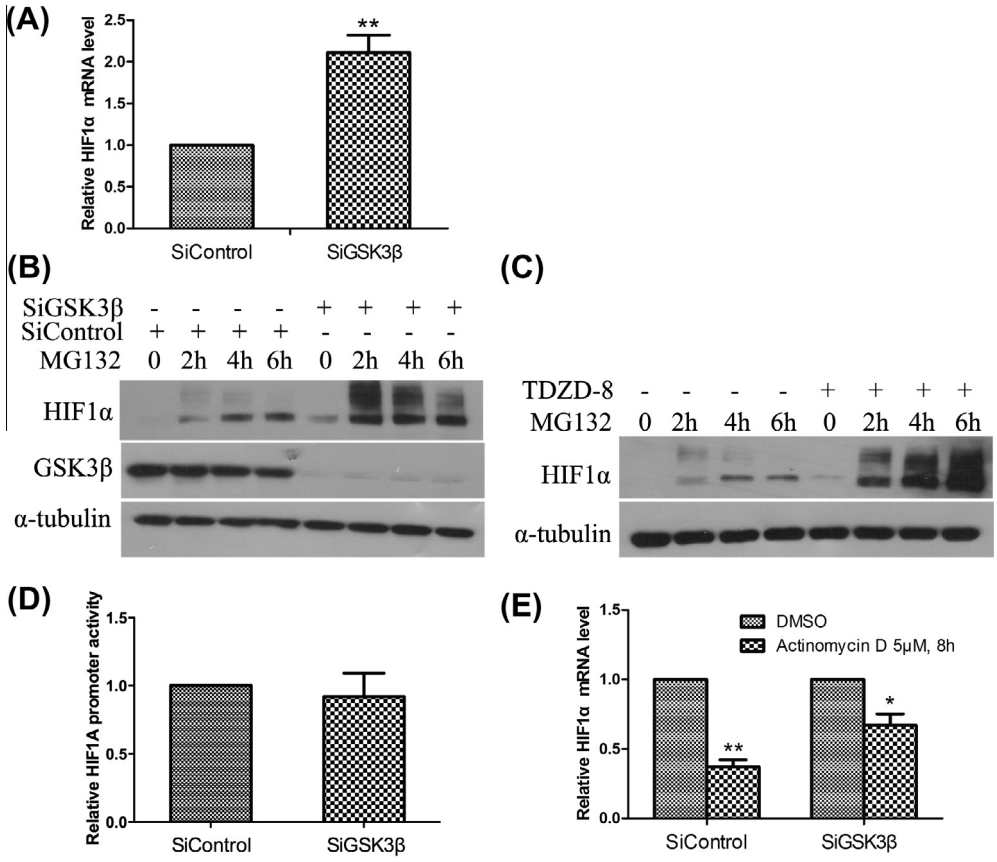

Fig. 3. Inhibition of GSK3β in osteosarcoma cells upregulated HIF1α mRNA expression and enhanced its mRNA stability. (A) Total RNA was isolated from SiControl- or SiGSK3β-treated cells. HIF1α mRNA expression was analyzed and quantified by real-time PCR as described in the Section 2. The relative level of HIF1α expression in the SiControl-treated cells was set at 1. $^{**}P < 0.01$. (B) The cells were infected with SiGSK3β or SiControl for the indicated times. (C) The cells were treated with the GSK3β inhibitor TDZD-8 at the indicated concentrations. (B, C) MG132 was used to block HIF1α protein degradation for the indicated times. The cells were lysed and equal amounts of proteins were separated and submitted to Western blot analysis using the indicated antibodies. (D) The HIF1A promoter region −572 to +284 was cloned into the pGL2 basic vector, which was transfected into MG63 cells. The cells were infected with SiGSK3β or SiControl, lysed and luciferase activity was measured. (E) The cells were infected with SiGSK3β or SiControl and then treated with actinomycin D for 8 h. HIF1α mRNA expression was analyzed and quantified by real-time PCR as described in the Section 2. The relative level of HIF1α expression in DMSO-treated cells was set at 1. $^{*}P < 0.05$.

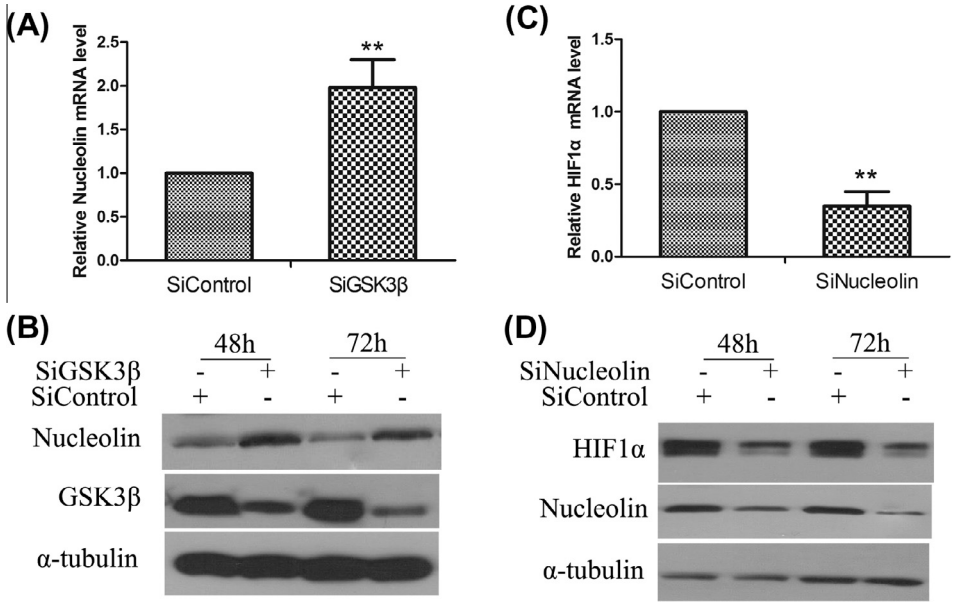

Fig. 4. Inhibition of GSK3β in osteosarcoma cells upregulated nucleolin mRNA expression. (A) The cells were infected with SiGSK3β or SiControl. Nucleolin mRNA expression was analyzed and quantified by real-time PCR as described in the Section 2. The relative level of nucleolin expression in SiControl-treated cells was set at 1. $^{**}P < 0.01$. (B) The cells were infected with SiGSK3β or SiControl for the indicated times. (C) The cells were infected with SiNucleolin or SiControl. HIF1α mRNA expression was analyzed and quantified by real-time PCR as described in the Section 2. The relative level of HIF1α expression in SiControl-treated cells was set at 1. $^{**}P < 0.01$. (D) The cells were infected with SiNucleolin or SiControl for the indicated times. (B, D) The cells were lysed and equal amounts of protein were separated and submitted to Western blot analysis using the indicated antibodies.

These data suggest that GSK3β affects HIF1α mRNA stability in MG63 cells via regulation of nucleolin expression.

4. Discussion

Expression of HIF1α is the main response to hypoxia and it is highly expressed in many tumors. HIF1a affects tumor angiogenesis, invasion and metastasis by activating a series of downstream proteins. However, the mechanism of HIF1α regulation under hypoxia is still controversial. It has been reported that TRAF6 can upregulate the expression of HIF1α and promote tumorigenesis [23]. Yeh et al. recently found that HEXIM1 reduced HIF1α protein stability, a process that is dependent on pVHL-mediated ubiquitination [24]. GSK3β is a serine/threonine kinase that phosphorylates glycogen synthase and is associated with many human diseases, especially the development and metastasis of a variety of malignant tumors [25]. GSK3β regulates tumor cell autophagy through the transcription factors CHOP and caspase-8 in MCF7 breast cancer cell lines [26]. Similarly, GSK3β is also an important oncogene and therapeutic target in endometrial carcinoma [13]. However, in lung cancer, GSK3β expression is low and it has been shown to act as a tumor suppressor gene and regulate tumor cell metastasis by phosphorylating Slug [27]. GSK3β is an important regulatory factor in nasopharyngeal carcinoma metastasis [28]. Celecoxib inhibits β-catenin by activating GSK3β to have an antitumor effect in osteosarcoma [29]. Currently, there are very few reports on the regulation of HIF1α by GSK3β in osteosarcoma in the literature. For the first time, we elaborate on the GSK3β-regulation of HIF1α in an osteosarcoma cell line at the protein and mRNA levels. Our results suggest that GSK3β negatively regulates HIF1α in the MG63 osteosarcoma cell line. This regulation does not occur by direct HIF1α protein phosphorylation inducing HIF1α protein degradation in a VHL independent manner but rather by the regulation of the stability of the HIF1α mRNA, which is a new type of regulating mechanism that has not been reported in the literature to date.

In our study, to verify the regulation of HIF1α by GSK3β in osteosarcoma, a GSK3β inhibitor or RNA interference was used to reduce the expression of GSK3β. Western blot analysis revealed that HIF1α protein expression levels were higher in these groups than in the control group, which was consistent with a previous report [19]. To explore the underlying mechanism, we used a small interfering RNA to inhibit levels of GSK3β, while excluding the impact of oxygen on HIF1α. The data showed that the HIF1α protein degradation rate did not change. To further verify this result, the Flag-HIF1α2Pm plasmid was constructed. The HIF1α protein stability produced by this plasmid was not affected by the oxygen content. Likewise, suppression or overexpression of GSK3β did not alter the degradation rate of HIF1α either. The above data showed us that GSK3β did not affect HIF1α protein stability in osteosarcoma cells. Using mutagenesis technology, Flag-HIF1α2Pm was mutated into Flag-HIF1α2PmSTSm (with S551A, T555V S589A mutations that could not be phosphorylated). The results showed that GSK3β had no effect on the degradation rate of HIF1α. Altogether, this means that GSK3β did not phosphorylate the three key amino acids in the ODD domain, resulting in the VHL-independent degradation of HIF1α, which implies that the regulation of HIF1α by GSK3β in osteosarcoma is via a distinct mechanism. Real-time PCR was used to detect the effect of GSK3β on HIF1α mRNA, and the experimental data indicated that inhibition of GSK3β promoted HIF1α mRNA expression. To verify this result, we used the protein degradation inhibitor MG132 to inhibit the degradation of HIF1α. Inhibition of GSK3β increased HIF1α accumulation. Therefore, GSK3β affected HIF1α mRNA levels. To further explore the role of GSK3β on the mechanism of HIF1α mRNA, we cloned the HIF1A promoter region −572 to +284 and used a luciferase assay to show that inhibition of GSK3β did not affect the HIF1A promoter activity. After inhibition of mRNA synthesis by actinomycin D, inhibition of GSK3β increased HIF1α mRNA stability. To sum up, GSK3β negatively regulated HIF1α protein by affecting its mRNA stability in the MG63 osteosarcoma cell line.

Nucleolin is a multifunctional phosphorylated protein that plays a critical role in DNA and RNA metabolism, and its expression is rather extensive. Nucleolin also participates in malignant tumors [30]. It has been shown that nucleolin binds to the mRNA of many important genes, including p53 and bcl-2, to affect mRNA translation [31]. Gonzalea et al. found that nucleolin could bind to the G-quadruplex structure in the 5′-UTR of the HIF1α mRNA [32]. For the first time, we have uncovered a role of nucleolin in the regulation of HIF1α in the MG63 osteosarcoma cell line. We found that inhibition of GSK3β could increase nucleolin protein and mRNA levels. Further experiments showed that inhibition of nucleolin could reduce HIF1α protein and mRNA levels, suggesting that regulation of HIF1α mRNA stability by GSK3β could be mediated through the regulation of nucleolin.

Current evidence suggests that in the MG63 osteosarcoma cell line, inhibition of GSK3β could increase HIF1α protein levels and the expression of its target genes by increasing the stability of the HIF1α mRNA, not by affecting the HIF1α protein stability, and that this process could be mediated by nucleolin. The manner by which GSK3β inhibition induces nucleolin expression remains to be elucidated.

Conflict of interest

None.

Acknowledgment

We are grateful the support of Dr. Jaime Caro for the kind suggestions and technical assistances.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.020.

References

[1] D.J. Manalo, A. Rowan, T. Lavoie, et al., Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1, Blood 105 (2005) 659–669.
[2] K. Hirota, G.L. Semenza, Regulation of angiogenesis by hypoxia-inducible factor 1, Crit. Rev. Oncol. Hematol. 59 (2006) 15–26.
[3] R.H. Wenger, D.P. Stiehl, G. Camenisch, Integration of oxygen signaling at the consensus HRE, Sci. STKE 2005 (2005) re12.
[4] G.L. Semenza, Hypoxia-inducible factor 1: master regulator of O2 homeostasis, Curr. Opin. Genet. Dev. 8 (1998) 588–594.
[5] G.L. Wang, B.H. Jiang, E.A. Rue, G.L. Semenza, Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 5510–5514.
[6] S. Salceda, J. Caro, Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes, J. Biol. Chem. 272 (1997) 22642–22647.
[7] M. Ivan, K. Kondo, H. Yang, et al., HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing, Science 292 (2001) 464–468.
[8] K. Tanimoto, Y. Makino, T. Pereira, L. Poellinger, Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein, EMBO J. 19 (2000) 4298–4309.
[9] D. Flügel, A. Görlach, T. Kietzmann, GSK-3β regulates cell growth, migration, and angiogenesis via Fbw7 and USP28-dependent degradation of HIF-1α, Blood 119 (2012) 1292–1301.
[10] N. Embi, D.B. Rylatt, P. Cohen, Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase, Eur. J. Biochem. 107 (1980) 519–527.
[11] J.R. Woodgett, Molecular cloning and expression of glycogen synthase kinase-3/factor A, EMBO J. 9 (1990) 2431–2438.
[12] T. Kang, Y. Wei, Y. Honaker, et al., GSK-3 beta targets Cdc25A for ubiquitin-mediated proteolysis, and GSK-3 beta inactivation correlates with Cdc25A overproduction in human cancers, Cancer Cell 13 (2008) 36–47.
[13] Y. Yin, N.T. Kizer, P.H. Thaker, et al., Glycogen synthase kinase 3β inhibition as a therapeutic approach in the treatment of endometrial cancer, Int. J. Mol. Sci. 14 (2013) 16617–16637.
[14] G. Manning, D.B. Whyte, R. Martinez, et al., The protein kinase complement of the human genome, Science 298 (2002) 1912–1934.
[15] B.W. Doble, J.R. Woodgett, GSK-3: tricks of the trade for a multi-tasking kinase, J. Cell Sci. 116 (2003) 1175–1186.
[16] R. Mishra, Glycogen synthase kinase 3 beta: can it be a target for oral cancer, Mol. Cancer 9 (2010) 144.
[17] H.T. Wu, S.Y. Ko, J.H. Fong, et al., Expression of phosphorylated Akt in oral carcinogenesis and its induction by nicotine and alkaline stimulation, J. Oral Pathol. Med. 38 (2009) 206–213.
[18] A. Iamaroon, S. Krisanaprakornkit, Overexpression and activation of Akt2 protein in oral squamous cell carcinoma, Oral Oncol. 45 (2009) e175–e179.
[19] D. Flügel, A. Görlach, C. Michiels, T. Kietzmann, Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner, Mol. Cell. Biol. 27 (2007) 3253–3265.
[20] X. Kong, B. Alvarez-Castelao, Z. Lin, et al., Constitutive/hypoxic degradation of HIF-alpha proteins by the proteasome is independent of von Hippel Lindau protein ubiquitylation and the transactivation activity of the protein, J. Biol. Chem. 282 (2007) 15498–15505.
[21] E. Minet, I. Ernest, G. Michel, et al., HIF1A gene transcription is dependent on a core promoter sequence encompassing activating and inhibiting sequences located upstream from the transcription initiation site and cis elements located within the 5′UTR, Biochem. Biophys. Res. Commun. 261 (1999) 534–540.
[22] D. Zhang, J. Li, M. Zhang, et al., The requirement of c-Jun N-terminal kinase 2 in regulation of hypoxia-inducing factor-1α mRNA stability, J. Biol. Chem. 287 (2012) 34361–34371.
[23] H. Sun, X.B. Li, Y. Meng, et al., TRAF6 upregulates expression of HIF-1α and promotes tumor angiogenesis, Cancer Res. 73 (2013) 4950–4959.
[24] I.J. Yeh, N. Ogba, H. Bensigner et al., HEXIM1 downregulates Hypoxia Inducible Factor-1 alpha protein stability, Biochem. J. (2013) Sep 10. [Epub ahead of print].
[25] B. Marchand, I. Tremblay, S. Cagnol, Boucher MJ, Inhibition of glycogen synthase kinase-3 activity triggers an apoptotic response in pancreatic cancer cells through JNK-dependent mechanisms, Carcinogenesis 33 (2012) 529–537.
[26] E. Gavilán, I. Sánchez-Aguayo, P. Daza, D. Ruano, GSK-3β signaling determines autophagy activation in the breast tumor cell line MCF7 and inclusion formation in the non-tumor cell line MCF10A in response to proteasome inhibition, Cell Death Dis. 4 (2013) e572.
[27] S.H. Kao, W.L. Wang, C.Y. Chen et al., GSK3β controls epithelial–mesenchymal transition and tumor metastasis by CHIP-mediated degradation of Slug, Oncogene (2013) Jul 15. [Epub ahead of print].
[28] R. Ma, Y. Wei, X. Huang, et al., Inhibition of GSK 3β activity is associated with excessive EZH2 expression and enhanced tumour invasion in nasopharyngeal carcinoma, PLoS ONE 8 (2013) e68614.
[29] J.J. Xia, L.B. Pei, J.P. Zhuang, et al., Celecoxib inhibits β-catenin-dependent survival of the human osteosarcoma MG-63 cell line, J. Int. Med. Res. 38 (2010) 1294–1304.
[30] S. Storck, M. Shukla, S. Dimitrov, P. Bouvet, Functions of the histone chaperone nucleolin in diseases, Subcell. Biochem. 41 (2007) 125–144.
[31] M. Takagi, M.J. Absalon, K.G. McLure, M.B. Kastan, Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin, Cell 123 (2005) 49–63.
[32] V. González, K. Guo, L. Hurley, D. Sun, Identification and characterization of nucleolin as a c-myc G-quadruplex-binding protein, J. Biol. Chem. 284 (2009) 23622–23635.