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Research Article

Dual-specificity phosphatase DUSP1 protects overactivation of hypoxia-inducible factor 1 through inactivating ERK MAPK

Changjiang Liu[1], Yongquan Shi[1], Yulei Du, Xiaoxuan Ning, Na Liu, Dawei Huang, Jie Liang, Yan Xue, Daiming Fan*

State Key Laboratory of Cancer Biology and Institute of Digestive Diseases, Xijing Hospital, the Fourth Military Medical University, Xi'an 710032, Shaanxi, China

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Abstract

Hypoxia-inducible factor 1 (HIF-1) plays a critical role in controlling oxygen delivery and metabolic adaptation to hypoxic conditions in hypoxic tumor cells. HIF-1 activation is initiated by several factors including mitogen-activated protein kinase (MAPK) superfamily. We have previously reported that mitogen-activated protein kinase phosphatase DUSP1 (MKP-1) was implicated in the negative regulation of HIF-1α subunit phosphorylation and HIF-1 activity. However, the molecular basis by which MKP-1 influences HIF-1 activity is not clarified. In this paper, we show that hypoxia transcriptionally induces MKP-1 expression in a time-dependent manner. Meanwhile, hypoxia also activates extracellular signal-regulated kinase (ERK) whose activity is enhanced or reduced by MKP-1 suppression or MKP-1 overexpression, respectively. We also show that suppression of MKP-1 expression facilitates the interaction between HIF-1α subunit and p300, a co-activator of HIF-1. Moreover, MKP-1 suppression leads to enhanced HIF-1 activity, which can be counteracted by PD98059, an ERK kinase inhibitor. Taken together, the results presented here suggest that hypoxia-induced MKP-1 protects overactivation of HIF-1 activation through inhibiting ERK kinase activity.

Keywords: Hypoxia; MKP-1; HIF-1; MAPK; Phosphorylation; Transactivation

Introduction

In hypoxia, several transcription factors were induced to respond to the decreased oxygen level. Among these transcription factors, hypoxia-inducible factor-1 (HIF-1) is one of the most important factors that play a critical role in controlling oxygen delivery and metabolic adaptation to hypoxic conditions [1–3]. HIF-1 is a heterodimer composed of two subunits, α and β. While both HIF-1α and HIF-1β are required to form the HIF-1 heterodimer, only HIF-1α is regulated by the oxygen tension. The regulation of HIF-1α occurs at posttranslational level involving modifications of hydroxylation, acetylation and phosphorylation.

In normoxic conditions, specific HIF-1α prolyl-hydroxylase hydroxylates two proline residues (P402 and P564) within the oxygen-dependent degradation domain of HIF-1α, resulting in HIF-1α recognition by von Hippel–Lindau protein ubiquitin ligase complex that targets HIF-1α for degradation through proteasome pathway [4–8]. Similarly, an asparaginyl hydroxylase termed as factor inhibiting HIF-1 hydroxylates the asparagine residue 803 within the trans-activation domain (TAD) of HIF-1α, abolishing its interaction with the transcriptional co-activator p300/CBP [9,10]. Hypoxia blocks both prolyl and asparaginyl hydroxylation, allowing HIF-1α to accumulate and bind to co-activator

Abbreviations: ERK, extracellular signal-regulated kinase; JNK, c-*jun* NH_2 terminal kinase; GST, glutathione *S*-transferase; HIF-1, hypoxia-inducible factor-1; HRE, hypoxia responsive element; MAPK, mitogen-activated protein kinase; MKP, mitogen-activated protein kinase phosphatase; siRNA, short interfering RNAs; TAD, trans-activation domain; VEGF, vascular endothelial growth factor.

* Corresponding author. Fax: +86 29 82539041.
E-mail address: fandaim@fmmu.edu.cn (D. Fan).

[1] These two authors contributed equally to this work.

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p300/CBP. In addition, HIF-1α acetylation by arrest defective-1 protein on lysine residue 532 shows increased interaction with von Hippel–Lindau protein and ubiquitinylation and subsequent degradation of HIF-1α. But in hypoxia, the amount of acetylated HIF-1α also diminished because of decreased arrest defective-1 protein expression [11].

HIF-1α regulation is not only associated with hydroxylation and acetylation, but also with phosphorylation [12–15]. It has been reported that there are two distinguishing forms of HIF-1α, phosphorylated and dephosphorylated, and only phosphorylated HIF-1α is able to combine HIF-1β to form a heterodimeric complex [16]. This fact indicated that only through phosphorylation could HIF-1 get activated. However, there is no direct evidence that shows HIF-1α phosphorylation enhances HIF-1 transcriptional activity. Recently, it has been reported that regulation mechanism of HIF-1 activity by extracellular signal-regulated kinase (ERK), a member of mitogen-activated protein kinase (MAPK) family, involves phosphorylation of co-activator p300, for the interaction between HIF-1α and p300/CBP is also ERK-dependent [17].

The active MAPK family members could be rapidly inactivated through dephosphorylation by phosphatases known as dual specificity mitogen-activated protein kinase phosphatases (MKPs) [18–20]. Among these phosphatases, MKP-1, encoded by an immediate early gene, showed equal efficacy in dephosphorylating all the three MAPK isoforms [18,19]. Recently, MKP-1 was identified as a hypoxia responsive gene, but the roles of which in the response of cells to hypoxia are poorly understood [21–23]. We have previously reported that MKP-1 was implicated in the negative regulation of HIF-1 activity [24]. Here we examined the molecular basis by which MKP-1 influences HIF-1 activity. Our results suggested that MKP-1 protects overactivation of HIF-1 activity through inactivating ERK activity.

Materials and methods

Cell culture

HepG2 cells were maintained in DMEM (Life Technologies) supplemented with 10% heat inactivated fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. For all the experiments, 12 h prior to normoxia or hypoxia incubation, the culture medium was replaced with serum-deprived DMEM to decrease serum-induced MKP-1 and ERK activation. For hypoxic exposure, cells were placed in a sealed chamber flushed with 1% O_2, 5% CO_2 and 94% N_2.

Plasmid constructs

Plasmid containing short interfering RNAs (siRNA) against MKP-1 was described previously [24]. Plasmid pGEX.H1α530-826 expressing glutathione *S*-transferase (GST)-TAD fusion protein (HIF-1α TAD contains p300 binding site.) was constructed by inserting PCR fragment into *Bam*HI and *Xho*I sites of pGEX-4T-1 (Amersham Biosciences). Plasmids expressing HIS-TAD (a fusion protein that contains HIS tag and HIF-1α TAD) or MKP-1 were constructed by inserting corresponding PCR fragments into pcDNA3.1 V5-His B (Invitrogen) at *Bam*HI and *Xho*I sites and pcDNA3.1/V5-His A (Invitrogen) at *Eco*RI and *Hin*dIII sites, respectively. The pGL3-SV40HRE containing two copies of erythropoietin hypoxia responsive element (HRE) was kindly provided by Prof. Mayuko Osada (Department of Chemical Biology, Osaka City University Medical School, Japan).

RT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen). About 1 μg of total RNA was used for first strand cDNA synthesis with kit according to the manufacturer's instructions. cDNAs were amplified using Ex Taq polymerase (TakaRa) with the following primer pairs. MKP-1, 5′-AGAACAGACAAAGAGCACCGCA-3′ and 5′-CCAATGGGATGTGAAGAGCCT-3′; HIF-1α, 5′-GCGGATCCATGGAATTCAAGTTGGAAT-3 and 5′-TCTCGAAACCTAGTTCAATTGGAGCTCGC-3′; β-actin, 5′-AGCGGGAAATCGTGCGTG-3′ and 5′-CAGGGTACATGG TGGTGCC-3′. The number of optimal replications was determined based on the linear correlations between cycle numbers and PCR products by running different number of PCR cycles. For MKP-1 and HIF-1α, 30 and 28 cycles were run, respectively. The PCR products were separated on ethidium bromide stained agarose, and visualized with UV.

Immunoprecipitation and Western blot analysis

Cells were lysed in lysis buffer. Lysates were first incubated with protein G sepharose (Invitrogen). The precleared lysates were incubated with 2 μg of monoclonal antibody against p300 on ice for 30 min followed by rocking with protein G sepharose at 4°C for 45 min. For Western blotting analysis, samples were resolved using SDS-PAGE and electrotransferred onto nitrocellulose membrane. The membranes were blocked in TBST (25 mM Tris, pH 7.5, 150 mM NaCl and 0.1% Tween 20) containing 5% dried fat-free milk for 2 h at room temperature, followed by overnight incubation at 4°C with primary antibody. Blots were washed three times in TBST and incubated for 1 h at room temperature with HRP-labeled secondary antibody in TBST. After extensive washes, the signals were visualized using ECL kit (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Transient transfection and reporter gene assay

Transient transfection was performed with FuGEN 6 reagents (Roche Applied Science) according to the serum-