Biochemical and Biophysical Research Communications 486 (2017) 270–276

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

p21-activated kinase 4 regulates HIF-1α translation in cancer cells

Hyunju Kim [a], Dustin J. Woo [a, b], So Yeon Kim [a, c, **], Eun Gyeong Yang [a, b, *]

[a] Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 02792, Republic of Korea
[b] Department of Biological Chemistry, KIST Campus, University of Science and Technology (UST), Seoul 02792, Republic of Korea
[c] Department of Biomedical Engineering, KIST Campus, University of Science and Technology (UST), Seoul 02792, Republic of Korea

ARTICLE INFO

Article history:
Received 27 February 2017
Accepted 8 March 2017
Available online 10 March 2017

Keywords:
Pak4
HIF-1α
Hypoxia
Akt
mTOR
4E-BP1

ABSTRACT

The p21-activated kinases (Paks) interact with Rac/Cdc42 GTPases to regulate the actin cytoskeleton as well as various signaling pathways. Although activation of Paks in many human cancers is known to mediate cancer progression, the role of Pak proteins in hypoxia is poorly understood. In this study, we found that both Pak1 and Pak4 are highly expressed in HeLa cervical cancer cells, but only Pak4 knockdown attenuates expression of hypoxia-inducible factor-1α (HIF-1α) in hypoxia. We further discovered that Pak4 regulates HIF-1α translation via the Akt-mTOR-4E-BP1 pathway under hypoxic conditions. These results support a novel connection between HIF-1α and Pak4 in hypoxic cancer cells, and provide insights into mechanisms whereby tumors respond to and thrive under oxygen-deficient conditions.

1. Introduction

The p21-activated kinases (Paks) belong to a family of serine/threonine kinases that act as effectors of the Rho-related GTPases such as Rac and Cdc42 [1]. Paks phosphorylate a diverse array of proteins regulating cytoskeletal remodeling as well as cell survival and motility. Since these processes are heavily involved in tumorigenesis where aberrant activation of Paks is commonly observed, much of the research on Pak proteins has focused on elucidating their molecular mechanisms in cancer progression [2–4].

The Pak family proteins are subdivided into two groups with Pak1-3 in group I and Pak4-6 comprising group II based on their sequences [1,5]. The two groups share a p21 binding domain and a C-terminal kinase domain but otherwise have distinct structural and functional features. Although the group I Paks have been better characterized so far, the group II Paks have recently been starting to garner some attention. Among the group II Paks, Pak4 is a ubiquitously expressed protein associated with embryonic development [6] and known to regulate actin cytoskeletal dynamics via the regulatory protein cofilin [7]. It is overexpressed or mutated in a variety of cancers including breast, colon, and cervical cancers [8–11]. The role of Pak4 in cancer has been linked to various cell signaling pathways such as the PI3K/AKT, ERK [8,12], NF-κB [9], and JNK [13] pathways.

Hypoxia-inducible factor (HIF) is the major transcription factor that elicits gene expression under hypoxic conditions. HIF-1α is a labile protein that is hydroxylated under normoxic conditions by the prolyl hydroxylase domain-containing proteins (PHDs), bound by the von-Hippel Lindau E3 ubiquitin ligase and subsequently degraded by the proteasome [14]. In hypoxia, PHD-catalyzed hydroxylation is abrogated due to a decreased oxygen concentration, and the stabilized HIF-1α then translocates to the nucleus where it forms a dimer with HIF-1β, thereby upregulating genes responsible for cell survival in oxygen-deficient conditions [14].

Possible connections between the Pak family proteins and hypoxia have been hinted at but not thoroughly investigated. For example, Pak1 down-regulation and subsequent HIF-1α up-regulation has been shown to alter cell morphology in pulmonary artery endothelial cells during pulmonary hypertension [15], but

Abbreviations: Pak, p21-activated kinase; HIF, hypoxia-inducible factor; PHD, prolyl hydroxylase domain-containing protein; FBS, fetal bovine serum; HRE, hypoxia response element; PGK-1, phosphoglycerate kinase-1; DFO, desferrioxamine; CHX, cycloheximide; 4E-BP1, 4E-binding protein 1; S6K, p70S6 kinase; S6, ribosomal S6 protein.

* Corresponding author. Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 02792, Republic of Korea.
** Corresponding author. Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 02792, Republic of Korea.
E-mail addresses: soyeonkim@kist.re.kr (S.Y. Kim), eunyang@kist.re.kr (E.G. Yang).

http://dx.doi.org/10.1016/j.bbrc.2017.03.024
0006-291X/

detailed signaling pathways responsible for HIF-1α upregulation have not been investigated. More recently, it was reported that Pak1 and HIF-1α form a positive feedback loop via the NF-κB pathway to regulate pulmonary vascular remodeling in muscle cells [16]. However, these results emphasize the roles of Pak1 in normal conditions, rather than in tumor environments. Given the prevalence of hypoxic regions in solid tumors, elucidation of links between Pak and HIF-1α in hypoxic cancer cells may further reveal mechanisms of cancer development. Employing siRNAs to silence endogenous Pak isoforms, we found that Pak4, but not Pak1, exhibits a stabilizing effect on the HIF-1α protein level in hypoxic cancer cells. By focusing on activities of protein kinases, we disclosed that Akt acts downstream of Pak4 to control HIF-1α translation by modulating the protein translation inhibitor 4E-BP1, uncovering a novel pathway in hypoxic cancer cells.

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS), DMEM, and RPMI were obtained from Life Technologies (Grand Island, NY). LY294002 was obtained from Cell Signaling Technology (Danvers, MA). Desferrioxamine (DFO), cycloheximide (CHX), MG132 and anti-β-actin antibody were obtained from Sigma-Aldrich (St. Louis, MO). Antibodies specific for Pak1, ERK1/2, phospho-ERK1/2, Akt, phospho-Akt, mTOR, phospho-mTOR, p70S6K, phospho-p70S6K, 4E-BP1, and phospho-4E-BP1 were obtained from Cell Signaling Technology. Anti-Pak4 antibody was purchased from Abcam (Cambridge, MA), and anti-HIF-1α antibody was from BD Bioscience (San Jose, CA).

2.2. Cell culture

Human cervical cancer cell line HeLa and colon cancer cell line HCT116 purchased from the American Type Culture Collection (Manassas, VA) were maintained in DMEM and RPMI supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C, respectively.

2.3. siRNA transfection and hypoxic induction

siRNA knockdown of Paks was performed using Lipofectamine RNAiMAX (Life Technologies Korea LLC) reagent as instructed. Negative control siRNAs (Bioneer Inc., Daejeon, Korea, no. SN-1003), Pak1 siRNAs (Bioneer Inc., no. 100282V), or Pak4 siRNAs (either no. 100272V from Bioneer Inc. or 5′-

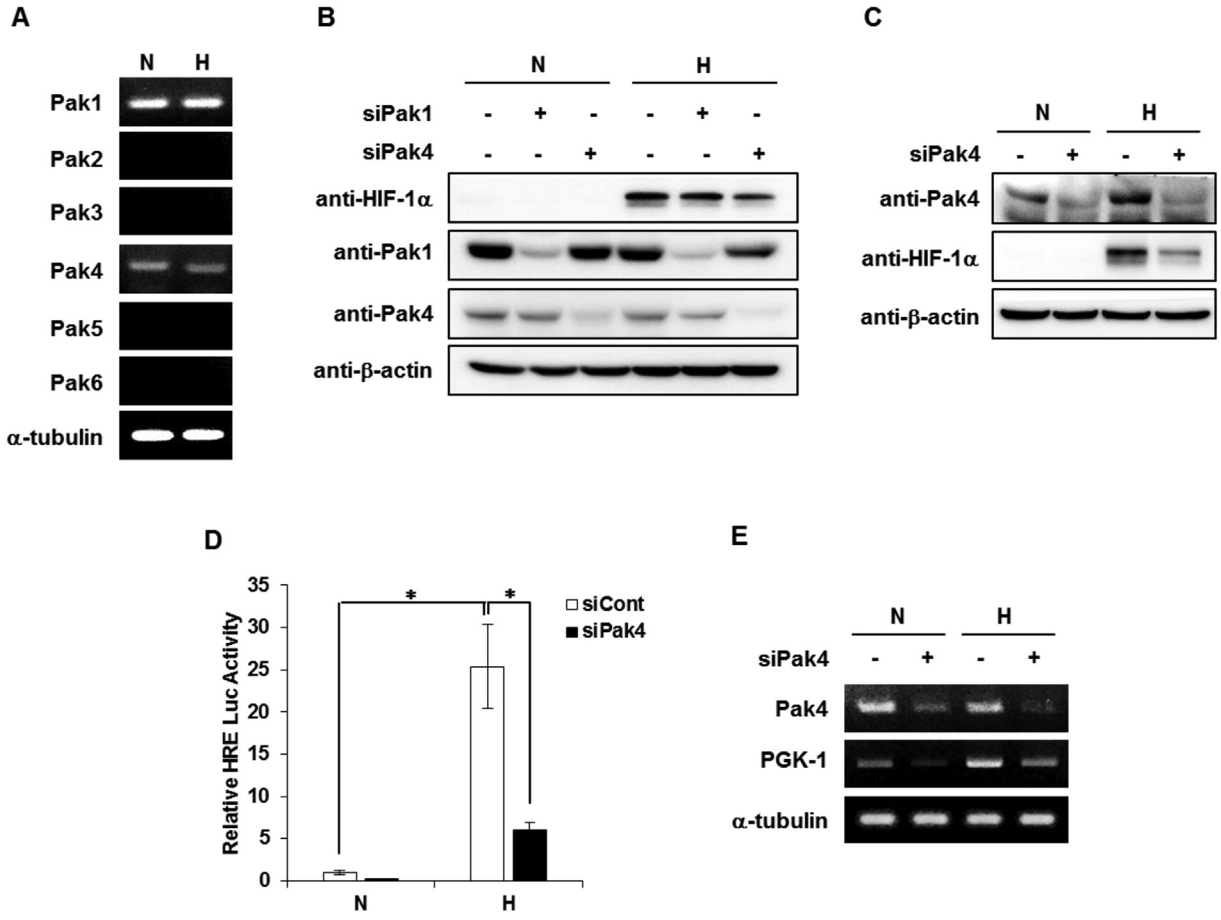

Fig. 1. Effect of knockdown of Pak4 on stabilization and activity of HIF-1α under hypoxic conditions. (A) mRNA levels of Pak isoforms (Pak1 ~ Pak6) in HeLa were measured by semi-quantitative RT-PCR (N, normoxia; H, hypoxia). (B) HeLa cells were transfected with control siRNAs, Pak1 or Pak4 siRNAs, and subjected to hypoxia for 18 h. The protein levels of HIF-1α, Pak1, and Pak4 were detected by Western blotting (N, normoxia; H, hypoxia). (C) HeLa cells were transfected with control siRNAs or second siRNAs targeting a distinct sequence within Pak4 gene, and followed by exposure to hypoxia for 18 h. HIF-1α and Pak4 were detected by Western blotting (N, normoxia; H, hypoxia). (D) HeLa cells were transfected with siRNAs targeting Pak4 or control siRNAs for 24 h, and then transfected with the HRE-Luc reporter plasmid for 24 h. The treated cells were exposed to hypoxic conditions for 18 h, harvested, and then subjected to a relative luciferase activity assay (N, normoxia; H, hypoxia). All quantitative data are presented as the mean ± S.D. of three independent experiments. *$P < 0.05$. (E) HeLa cells were transfected with siRNAs targeting Pak4 for 24 h and then exposed to hypoxic conditions for 18 h. The mRNA level of PGK-1 was determined by semi-quantitative RT-PCR (N, normoxia; H, hypoxia).