Biomedicine & Pharmacotherapy 77 (2016) 176–181

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

HPIP promotes non-small cell lung cancer cell proliferation, migration and invasion through regulation of the Sonic hedgehog signaling pathway

Jia Pan[1,*], Yaguang Qin[1], Mengwei Zhang

Department of Oncology, Huaihe Hospital of Henan University, Kaifeng 475000, PR China

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 2 December 2015
Accepted 15 December 2015

Keywords:
Hematopoietic pre-B cell leukemia transcription factor (PBX)-interacting protein (HPIP)
Non-small cell lung cancer (NSCLC)
Cell proliferation
Invasion

ABSTRACT

Hematopoietic pre-B-cell leukemia transcription factor (PBX)-interacting protein (HPIP) has been shown to play a role in cancer development and progression. However, the role of HPIP in non-small cell lung cancer (NSCLC) has never been revealed. Here, we explore the roles and mechanisms of HPIP in the progression of NSCLC. Our results showed that HPIP expression was significantly higher in NSCLC tissues and cell lines when compared with that in normal lung tissues and cell line. In addition, knockdown of HPIP in NSCLC cells significantly inhibits the proliferation, migration and invasion *in vitro* and tumor growth *in vivo*. Furthermore, knockdown of HPIP obviously inhibits the protein expression of Shh as well as Smo, Ptc and Gli-1 in A549 cells. Taken together, these results strongly suggest that knockdown of HPIP inhibited NSCLC cell proliferation and invasion through suppressing the Sonic hedgehog signaling pathway. Thus, HPIP may be a novel potential therapeutic target for NSCLC.

1. Introduction

Lung cancer is the most common type of cancers and the leading cause of cancer-related mortality worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases [1,2]. In recent years, despite recent advances in clinical and experimental oncology [3–5], the prognosis of NSCLC remains very poor with the 5-year survival rate a dismal 11% [6]. Thus, further elucidation of the molecular mechanisms underlying NSCLC may have a significant impact on the systematic treatment of this disease.

Hematopoietic pre-B-cell leukemia transcription factor (PBX)-interacting protein (HPIP/PBXIP1), a corepressor for the transcription factor PBX, is a nucleo-cytoplasmic shuttling protein [7]. It is also known that HPIP interacts with estrogen receptor by associating with the microtubule network, and recruits Src kinase and the p85 subunit of phosphatidylinositol 3-kinase (PI3K) to estrogen–estrogen receptor complex, which in turn activates AKT and ERK1/2 [8]. Recently, emerging studies have indicated that HPIP expression is very low in normal cells, while its expression is increased in many tumors, including gastric cancer, colorectal cancer, breast infiltrative ductal carcinoma and astrocytoma, and HPIP affects adhesion, migration, and invasion of tumor cells, and thereby contributes to tumorigenesis and metastasis of malignant tumors *in vivo* [9–12]. However, the role of HPIP in NSCLC has never been revealed. Here, we explore the roles and mechanisms of HPIP in the progression of NSCLC. Our results suggested that high expression of HPIP might be involved in NSCLC carcinogenesis.

2. Materials and methods

2.1. Sample collection

Fresh tumor tissues and adjacent normal tissues from 14 well-established primary NSCLC patients were collected in the Department of Oncology, Huaihe Hospital of Henan University (China) from 2013 to 2014. 11 patients were men while 3 were women, and their average age was 64 years (range, 41–82). NSCLC tissues were classified as well (grade I; $n=1$), moderately (grade II; $n=8$), or poorly (grade III; $n=5$). The stages of the NSCLC patients were evaluated by TNM staging (stage I, 2 patients; stage II, 5 patients; stage III, 5 patients and stage IV, 2 patients). No patients had received blood transfusion, radiotherapy, or chemotherapy before surgery. The samples were immediately stored in liquid nitrogen in preparation for use. This study was approved by the

* Corresponding author at: Department of Oncology, Huaihe Hospital of Henan University, No 115, Ximen Avenue, Kaifeng 475000, P.R. China.
Fax: +86 037123906821.
E-mail address: oncol_jp@163.com (J. Pan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biopha.2015.12.012
0753-3322/

Ethnic Committee of Huaihe Hospital of Henan University, and written informed consent was obtained from all participants.

2.2. Cell culture

Three human NSCLC cell lines (A549, H1299 and 95-D) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). A normal human bronchial epithelial cell line (HBE), were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37° C with 5% CO_2 in an incubator (Life Technologies, Baltimore, MD, USA).

2.3. RNA interference and transfection

HPIP small interfering RNAs (siRNA-HPIP) and its negative control were synthesized by RiBo Biotech (GuangZhou, RiBo Biotech). For transfection, cells were seeded in 24-well plates in triplicate containing DMEM supplemented with 10% FBS. The cells were transfected with siRNA-HPIP or the control siRNA (mock) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions.

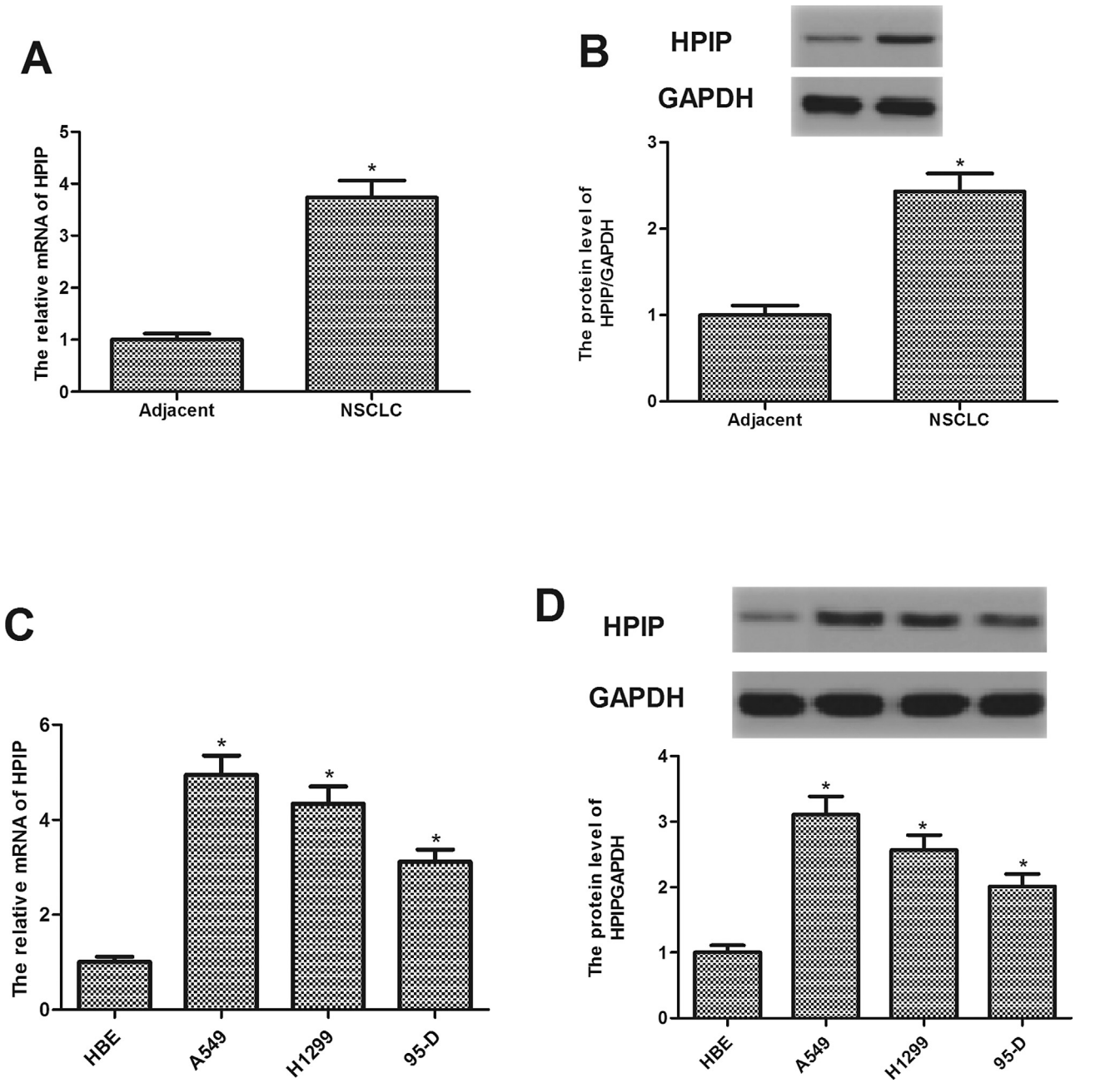

Fig. 1. HPIP is up-regulated in NSCLC. (A) qRT-PCR analysis of HPIP mRNA in 14 NSCLC tissues and paired adjacent normal lung tissues. HPIP mRNA levels in human NSCLC tissues were obviously higher than that in normal lung tissues; (B) Western blot analysis of HPIP protein in the indicated samples. HPIP protein was also significantly up-regulated in human NSCLC tissues; (C) The representative mRNA expression of HPIP in human NSCLC cell lines; (D) The representative western image of HPIP protein in human NSCLC cell lines. Each column represents mean ± SD; $n = 3$ per group. $^{*}p < 0.05$.