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Original article

HUWE1 upregulation has tumor suppressive effect in human prostate cancer cell lines through c-Myc

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ABSTRACT

Purpose: We investigated the regulatory function of HECT, UBA and WWE domain-containing protein 1, E3 ubiquitin protein ligase (HUWE1) in human prostate cancer (CaP).

Methods: HUWE1 was overexpressed (through transfection) or downregulated (through lentiviral transduction) in CaP cell lines, PC3 and DU145 cells. The functions of HUWE1 overexpression or downregulation on CaP cancer cell proliferation, migration*in vitro*, and explant *in vivo* were examined. In addition, the regulatory effect of HUWE1 on c-Myc expression was assessed. In HUWE1-overexpressed CaP cells, c-Myc was further upregulated to assess whether c-Myc was directly involved in HUWE1-induced regulation in CaP.

Results: HUWE1 overexpression inhibited CaP proliferation and migration*in vitro*, and explant growth *in vivo*. On the other hand, HUWE1 downregulation had no effects on CaP *in vitro*. C-Myc was downregulated in HUWE1-overexpressed, but un-changed in HUWE1-downregulated, CaP cells. Further upregulating c-Myc in HUWE1-overexpressed CaP cells reversed the tumor-suppressing effects by HUWE1-overexpression on cancer proliferation and migration *in vitro*.

Conclusion: HUWE1 overexpression could functionally suppress CaP development both*in vitro* and *in vivo*, possibly by inverse regulation on c-Myc.

1. Introduction

Prostate cancer (CaP) is one of the most diagnosed cancers among male patients, especially in developed countries [1,2]. According to recent studies, CaP is the second leading cause of cancer death in men in the United States [3,4]. By 2011, there were more than 2.5 million CaP survivors in the states [3]. This number increased to approximate 3 million in 2017 [4]. In developing countries, such as in China, prostate cancer is also one of the most commonly diagnosed and most deadly male cancers [5,6]. In the recent decade, the incidence rate of CaP has been constantly rising in China [6]. According to a recent report, the overall incidence of CaP was 7.1/105 populations in 2011, ranked ninth in the highest cancer incidences for all sexes and seventh in male [5,6]. Moreover, there has been no standard procedure in China on CaP screening, due to lack of China-based cancer patient data and the concern of overly diagnostic or treatment approaches [5].

Recently, signaling pathways associated with WWE domain-containing protein 1, E3 ubiquitin protein ligase (HUWE1) have been demonstrated to play crucial roles in human cancers, including tumorigenesis, tumor maturation, metastasis and apoptosis [7]. However, the exact mechanisms of HUWE1 in human cancer seem controversial. In 2005, two studies reported completely opposite phenotypes of hwue1-knockout in human cancer cells in the same issue of the journal of CELL [8,9]. In addition, HUWE1 was discovered to be aberrantly overexpressed in human lung cancer and breast cancer, acting as an oncogene [10,11], but downregulated in human thyroid cancer and colorectal cancer, and acting as a tumor suppressor [12,13]. In human prostate cancer, histone demethylase JMJD1A was demonstrated to modulate proto-oncogene c-Myc by inhibiting HUWE1-mediated c-Myc degradation, thus suggesting that HUWE1 might be a tumor suppressor in human CaP [14]. However, the direct evidence HUWE1-initiated regulation in human prostate cancer has never been reported.

In this study, we applied genetic modification technology to either overexpress or downregulate HUWE1 in *in vitro* human CaP cell lines, PC3 and DU145 cells, thus to explore the mechanistic functions of HUWE1 upregulation or downregulation on CaP cancer development, including proliferation and migration *in vitro* and tumor explant growth

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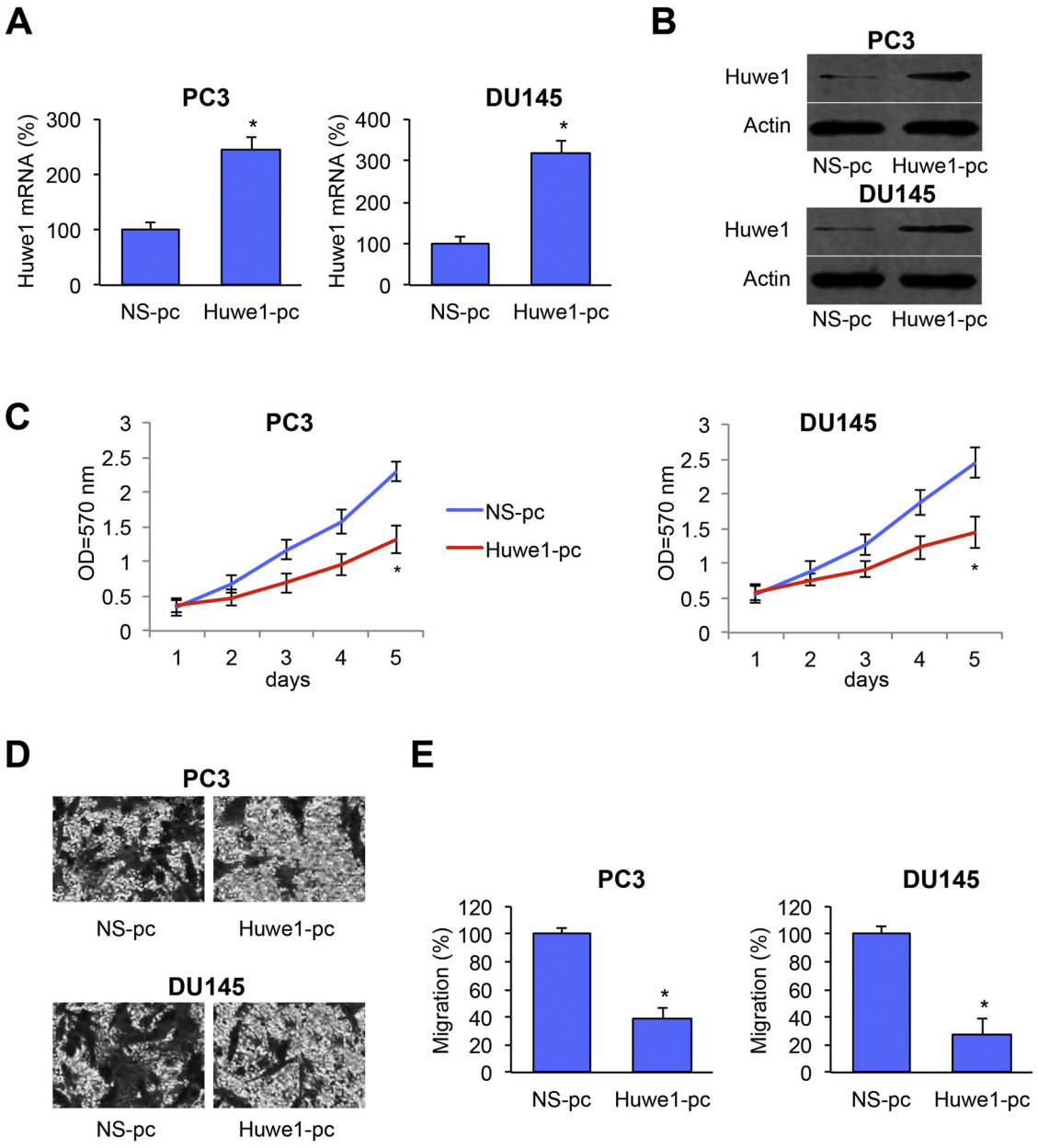

Fig. 1. Overexpressing HUWE1 suppressed CaP *in vitro*. (A) PC3 and DU145 cells were transfected with a HUWE1 overexpression plasmid, HUWE1-pc, or a non-specific overexpression plasmid, NS-pc. Post transfection, qRT-PCR was used to compare endogenous HUWE1 mRNA levels between NS-pc- and HUWE1-pc-transfected cancer cells (* $P < 0.05$). (B) Post transfection, western blot was used to compare HUWE1 protein expressions between NS-pc- and HUWE1-pc-transfected PC3 and DU145 cells. (C) Transfected PC3 and DU145 cells were re-seeded in 96-well plates for 5 days. An *in vitro* proliferation assay was conducted. Cancer cell daily proliferating rates were measured at optical density (OD) of 570 nm (* $P < 0.05$, one-way ANOVA). (D) Transfected PC3 and DU145 cells were re-plated in the inserts of a 24-well transwell for 24 h. An *in vitro* migration assay was conducted. PC3 and DU145 cells successfully crossed the insert barrier and reached the bottoms of transwell were shown. (E) Within migration assay, relative migration was measured and compared between NS-pc- and HUWE1-pc-transfected PC3 and DU145 cells (* $P < 0.05$).

in vivo. In addition, in HUWE1-overexpressed PC3 and DU145 cells, we modulated the expression of HUWE1-associated proto-oncogene, c-Myc, to further explore the downstream signaling pathways of HUWE1 in regulating CaP cancer cell development.

2. Materials and methods

2.1. Ethic statement

This study was approved by the Clinical Study and Ethics Committees at Shandong Provincial Hospital affiliated to Shandong University in Jinan and Chinese PLA 252 Hospital in Baoding, China. All protocols in this study were performed in accordance with the World Medical Association Declaration of Helsinki in 2013 [15].

2.2. Human prostate cancer cell lines

Human prostate cancer (CaP) cell lines, PC3 and DU145 cells were commercially obtained from American Type Culture Collection (ATCC, USA). Both cells were maintained in 6-well tissue culture plates (Jet Biofil, Japan) in RPMI -1640 medium (MilliporeSigma, Shanghai, China) supplemented with 10% fetal bovine serum (10% FBS, MilliporeSigma, Shanghai, China) and 1% V/V penicillin/streptomycin (MilliporeSigma, Shanghai, China), at 37 °C in a humidified

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