ARTICLE
Journal of Cellular Biochemistry 115:1762–1767 (2014)

Journal of Cellular Biochemistry

Downregulation of Homeodomain-Interacting Protein Kinase-2 Contributes to Bladder Cancer Metastasis by Regulating Wnt Signaling

Mingyue Tan, Hua Gong, Yigang Zeng, Le Tao, Jun Wang, Juntao Jiang, Dongliang Xu, Erdun Bao, Jianxin Qiu,* and Zhihong Liu*

Department of Urology, Shanghai First People's Hospital, Medical College of Shanghai Jiao Tong University, Shanghai, China

ABSTRACT

Homeodomain-interacting protein kinase-2 (Hipk2) has been shown to have important regulatory roles in cancer biology, such as cancer cell proliferation, cell cycle, and cell invasion. However, the contributions of Hipk2 to bladder cancer metastasis remain largely unknown. In the current study, we assayed the expression level of Hipk2 in bladder cancer tissues by real-time PCR, and defined its biological functions. We found that Hipk2 levels were downregulated in most bladder cancer tissues compared with adjacent normal tissues, and Hipk2 levels were remarkably decreased in metastasized tumor tissues when compared with primary tumors. SiRNA-mediated Hipk2 silencing increased bladder cancer cell invasion. Hipk2 knockdown resulted in decrease of E-cadherin expression and increase of N-cadherin and fibronectin expression, indicated that epithelial-mesenchymal transition (EMT) was induced. We further demonstrated that Hipk2 knockdown induced Wnt signaling activation and β-catenin nuclear localization. Finally, we confirmed that Hipk2 inhibition promoted EMT and subsequent cell invasion, at least in part by activating Wnt signaling. These data suggest an important role of Hipk2 in regulating metastasis of bladder cancer and implicate the potential application of Hipk2 in bladder cancer therapy. J. Cell. Biochem. 115: 1762–1767, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: Hipk2; Wnt; EMT; BLADDER CANCER; METASTASIS

Human bladder cancer is the fourth most common malignancy in men, and the tenth most common in women [Lacey et al., 2002; Liu et al., 2013]. The most common histological type of bladder cancer is urothelial carcinoma (UC) which are non-invasive papillary tumors that commonly recur but rarely progress [Seiler et al., 2012]. Metastatic bladder tumors are more aggressive, and patients with muscle invasive UC are usually treated with radical cystectomy. However, one-half of patients with invasive bladder cancer develop subsequent metastatic disease, even after radical surgery of the primary tumors [Said et al., 2012]. The advances in effective therapy for these patients have been limited because the pathological mechanisms causing tumor are not known. Therefore, revealing the molecular mechanism for the bladder cancer metastasis is indispensable for developing effective treatment.

The initial stage of metastatic progression is essentially dependent on the prominent biological event referred to as epithelial–mesenchymal transition (EMT), which is characterized by specific morphogenetic changes, loss of cell adhesion, and increased cell movement [Cai et al., 2013]. Loss of E-cadherin expression is a hallmark of the EMT process and is likely required for enhanced tumor cell motility [Chua et al., 2007; Ying et al., 2012]. Wnt/β-catenin signaling has been shown to play an important role in the regulation of EMT and cancer metastasis [Anson et al., 2012; Cai et al., 2013]. Wu et al. [2012] showed that canonical Wnt

Abbreviations used: Hipk2, homeodomain-interacting protein kinase-2; EMT, epithelial–mesenchymal transition; UC, urothelial carcinoma.

Mingyue Tan and Hua Gong contributed equally to this work.

The authors declared that they have no conflicts of interest.

Grant sponsor: Shanghai Municipal Nature Science Fund; Grant number: 13ZR1433100.

*Correspondence to: Zhihong Liu or Jianxin Qiu, Department of Urology, Shanghai First People's Hospital, Medical College of Shanghai Jiao Tong University, No. 100 Haining Road, Shanghai 200080, China. E-mail: drzhihongliu@gmail.com or qiujason@sh163.net

Manuscript Received: 17 December 2013; Manuscript Accepted: 9 May 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 13 May 2014

DOI 10.1002/jcb.24842 • © 2014 Wiley Periodicals, Inc.

suppressor, Axin2, acts as a potent promoter of carcinoma behavior by increasing the activity of the Snail1, which induces a functional EMT and drives metastatic activity. Axin2 inhibition decreases Snail1 activity, reverses EMT, and inhibits colorectal cancer invasion and metastasis [Wu et al., 2012].

Homeodomain-interacting protein kinase-2 (Hipk2) is a serine/threonine kinase that as been shown to be involved in tumor suppressor [Wei et al., 2007]. Hipk2 is activated in response to various types of DNA-damaging agents, and activated Hipk2 phosphorylates p53 for specific activation of proapoptotic target genes and contributes to the regulation of p53-induced apoptosis [Puca et al., 2008]. Recent studies reveal that Hipk2 regulates cancer metastasis [Nodale et al., 2012]. Nodale et al. [2012] showed that Hipk2 downregulates vimentin expression in invasive MDA-MB-231 breast cancer cells and in the non-invasive MCF7 breast cancer cells subjected to chemical hypoxia. They further demonstrated that vimentin downregulation by Hipk2 correlates with inhibition of breast tumor cell invasion. Hipk2 may control cancer metastasis by regulating Wnt/β-catenin. Wei et al. [2007] demonstrated that Hipk2 represses β-catenin-mediated transcription and epidermal stem cell expansion. They showed that the C-terminus of Hipk2 is required to interact with the corepressor, CtBP, which in turn promotes the suppressor function of Hipk2 [Zhang et al., 2005; Wei et al., 2007]. Deletion of the YH domain and the putative CtBP binding motif of Hipk2 completely abolish the repressor effect of Hipk2 in TOPFlash reporter assays. However, little is known about whether dysregulation of Hipk2 regulates bladder cancer metastasis by regulating Wnt signaling.

Based on these findings, we investigated whether Hipk2 regulates Wnt/β-catenin activation and subsequent bladder cancer metastasis. We found that Hipk2 expression levels were downregulated in metastasized tumor tissues. Decreased expression of Hipk2 activated the Wnt pathway to promote EMT and human bladder cancer cell metastasis.

MATERIALS AND METHODS

TISSUE SAMPLES AND CELL LINES

Human bladder tissues were obtained with informed consent from the First People's Hospital affiliated to School of Medicine Shanghai Jiaotong University. The protocols used in the study were approved by the Hospital's Protection of Human Subjects Committee. Twenty-five specimens of normal and pathologically diagnosed biopsy specimens were obtained from patients with bladder tumors, including 18 with lymph node metastasis and 7 without (median age 61, range 52–79). Human bladder cancer cells T24 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and were maintained in RPMI 1640 with 10% FBS (GIBCO, Carlsbad, CA), and were cultured at 37°C with 5% CO_2.

REAL-TIME POLYMERASE CHAIN REACTION

Total RNA was extracted from bladder cancer tissues or cells by using Trizol reagent (Invitrogen, Carlsbad, CA), and the reverse transcription reactions were performed using an M-MLV Reverse Transcriptase kit (Invitrogen). Real-time PCR was carried out using a standard protocol from the SYBR Green PCR kit (Toyobo, Osaka, Japan) on Applied Biosystems 7300 Real Time PCR system (Applied Biosystems, Foster City, CA) according to the instructions. β-actin was used as references for mRNA. ΔCt values were normalized to β-actin levels. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative quantitation of gene expression levels.

WESTERN BLOT ANALYSIS

Western blot analysis to assess Hipk2, E-cadherin, N-cadherin, fibronetic, and β-actin expression was carried out as previously described [Kapinas et al., 2007]. The Hipk2, E-cadherin, N-cadherin, fibronetic primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). β-actin primary antibodies were purchased from Sigma (MO).

LUCIFERASE REPORTER ASSAY

To test Wnt signaling, T24 cells were cotransfected with either the Wnt signaling reporter TOPFlash or the negative control FOPFlash along with the Hipk2-siRNA (Santa Cruz, CA) as previously described [Tano et al., 2010]. The siRNAs used in this study were mixtures of three siRNAs as previously described [Pierantoni et al., 2011]. Transfected T24 cells were then treated with Wnt3a. The data are represented as normalized TOPFlash/FOPFlash values.

IMMUNOFLUORESCENCE STAINING

Immunofluorescence staining was carried out as described previously [Si et al., 2006]. Briefly, T24 cells were fixed with methanol and washed with PBS. Then the fixed cells were permeabilized with 1% NP-40 and blocked with calf serum, followed by incubating cells with an anti-β-catenin antibody (Santa Cruz, CA) for 1 h at room temperature. After being washed, the cells were incubated with biotin-labeled secondary antibody for 30 min, followed by incubating the cells with streptavidin-Alexa Fluor 594 (Molecular Probes, Eugene, OR) for 20 min. The presence of β-catenin protein was visualized under a fluorescence microscope.

TRANSWELL INVASION ASSAY

Cell invasion was administrated using Transwell invasion assay with inserts of 8-μm pore size (Corning Costar) as described previously [Connor et al., 2007]. Briefly, T24 cells were suspended in medium without serum and seeded onto Matrigel-coated Transwell filters in Biocoat Matrigel invasion chambers. Medium supplemented with serum was used as a chemo-attractant in the lower chamber. The cells were treated with Hipk2-siRNA for 72 h, and cells that did not invade through the pores were removed using a cotton swab. Cells on the lower surface of the membrane were fixed with methanol and stained with Crystal violet. The cell numbers were determined by counting of the penetrating cells under a microscope at 200× magnification in random fields in each well. Each experiment was performed in triplicate.

STATISTICAL ANALYSIS

All data are expressed as mean ± standard deviation (SD) from at least three separate experiments. The differences between groups were analyzed using Student's *t*-test. The difference was deemed statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Hipk2 IS ASSOCIATED WITH BLADDER CANCER METASTASIS

To investigate the role of Hipk2 in bladder cancer progression, we first assayed the expression level of Hipk2 in bladder cancer tissues. As shown in Figure 1A, Hipk2 expression levels were significantly downregulated in most bladder cancer tissues compared with adjacent normal controls. Hipk2 was decreased expression in 80% (20/25) of bladder cancer in comparison with adjacent normal control (Fig. 1A). When these tumor tissues were stratified based on clinical progression, we found that the Hipk2 levels are lower in primary tumors that subsequently metastasized than those in non-metastatic bladder cancer (Fig. 1B). We next examined the Hipk2 expression level in the primary and lymph-node metastatic tumor specimen by quantitative RT-PCR. Expression analysis of Hipk2 in 18 primary bladder cancer tissues and the corresponding lymph-node metastases pairs, each from the same patient, showed markedly lower Hipk2 levels in the metastases of all 18 pairs (Fig. 1C). These data indicate that downregulation of Hipk2 may be related to bladder cancer metastasis.

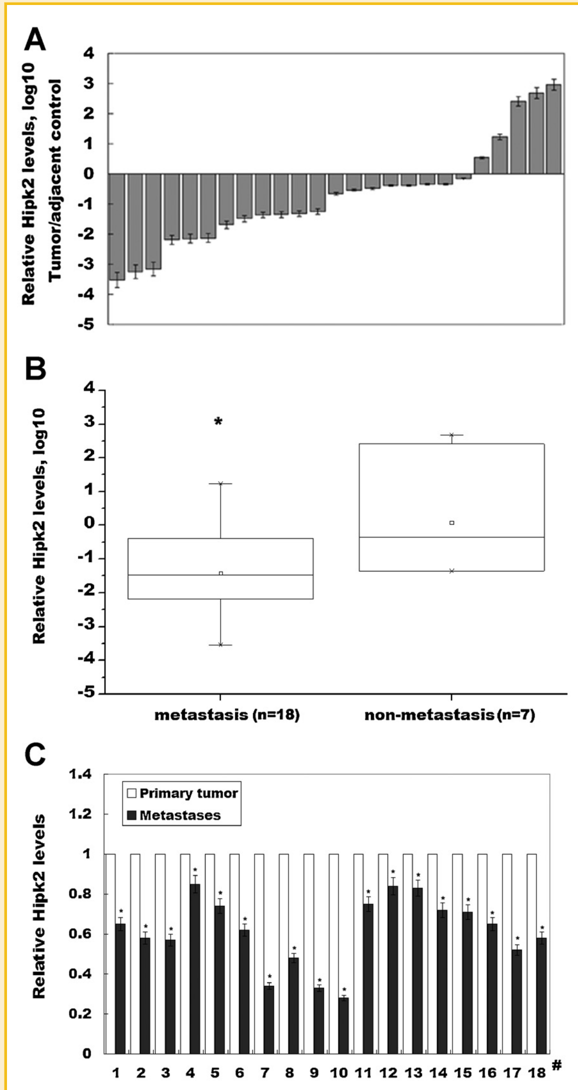

Fig. 1. Hipk2 is associated with bladder cancer metastasis. A: The analysis of Hipk2 expression level was performed in bladder tumor tissues (n = 25) and matched normal tissues. Total RNA was subjected to quantitative RT-PCR to analyze the expression level of Hipk2 in each sample. β-actin was used as a reference. The relative Hipk2 levels were calculated by $2^{-\Delta\Delta Ct}$ where $\Delta Ct = Ct$ (Hipk2) − Ct (β-actin) and $\Delta\Delta Ct = \Delta Ct$ (tumor tissue) − ΔCt (adjacent normal tissue). B: The tumor samples were divided into two groups based on clinical progression. The Hipk2 expression levels in the metastasis group (n = 18) and in the no-metastasis group (n = 7) were assayed. $^*P < 0.05$. C: The Hipk2 expression in 18 primary and corresponding lymph-node metastatic tumor specimen was analyzed by quantitative RT-PCR. β-actin was used as a reference. Data are expressed as mean ± SD from at least three separate experiments. The differences between groups were analyzed using Student's *t*-test.

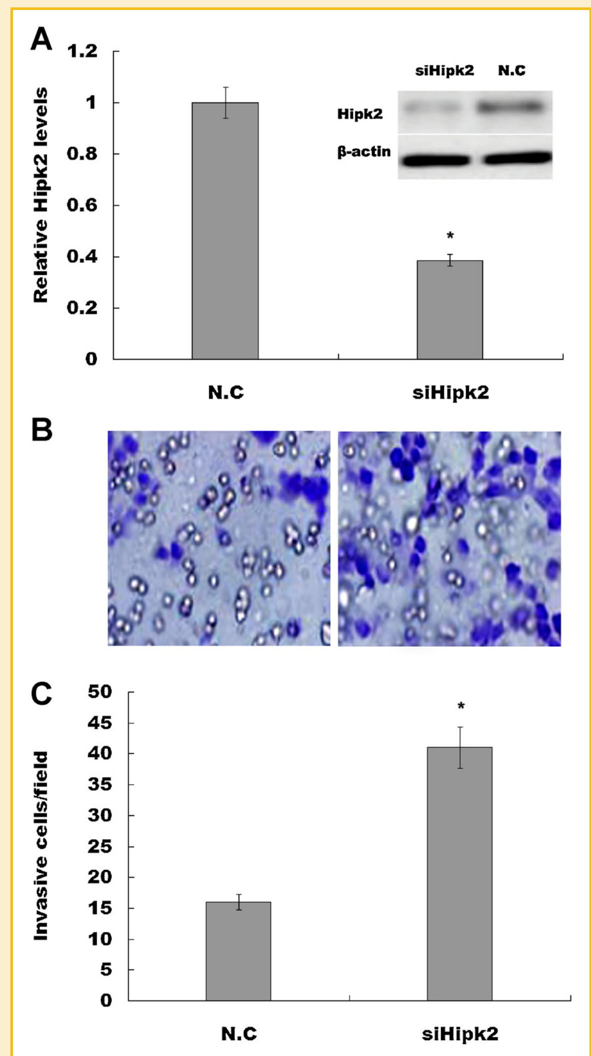

Fig. 2. Hipk2 knockdown promotes bladder cancer cell invasion. A: The Hipk2 mRNA and protein expression was significantly decreased in T24 cells treated with Hipk2-siRNA. N.C, negative control. B,C: Hipk2 knockdown promoted T24 cell invasion. Profiles are representative of at least three independent experiments. Statistical analysis is shown at the bottom (C). $^*P < 0.05$.

Hipk2 SILENCING PROMOTES BLADDER CANCER CELL INVASION

To further study the role of Hipk2 in cell invasion, the bladder cancer cells treated with Hipk2-siRNAs were analyzed. We first proved that T24 cells can be used as an in vitro model to investigate Hipk2 function by assaying the Hipk2 expression in T24 cells (Fig. 2A). The Hipk2 mRNA and protein levels were significantly decreased in T24 cells treated with Hipk2-siRNAs (Fig. 2A). siRNA-mediated Hipk2 silencing increased bladder cancer cell invasion in vitro (Fig. 2B,C).

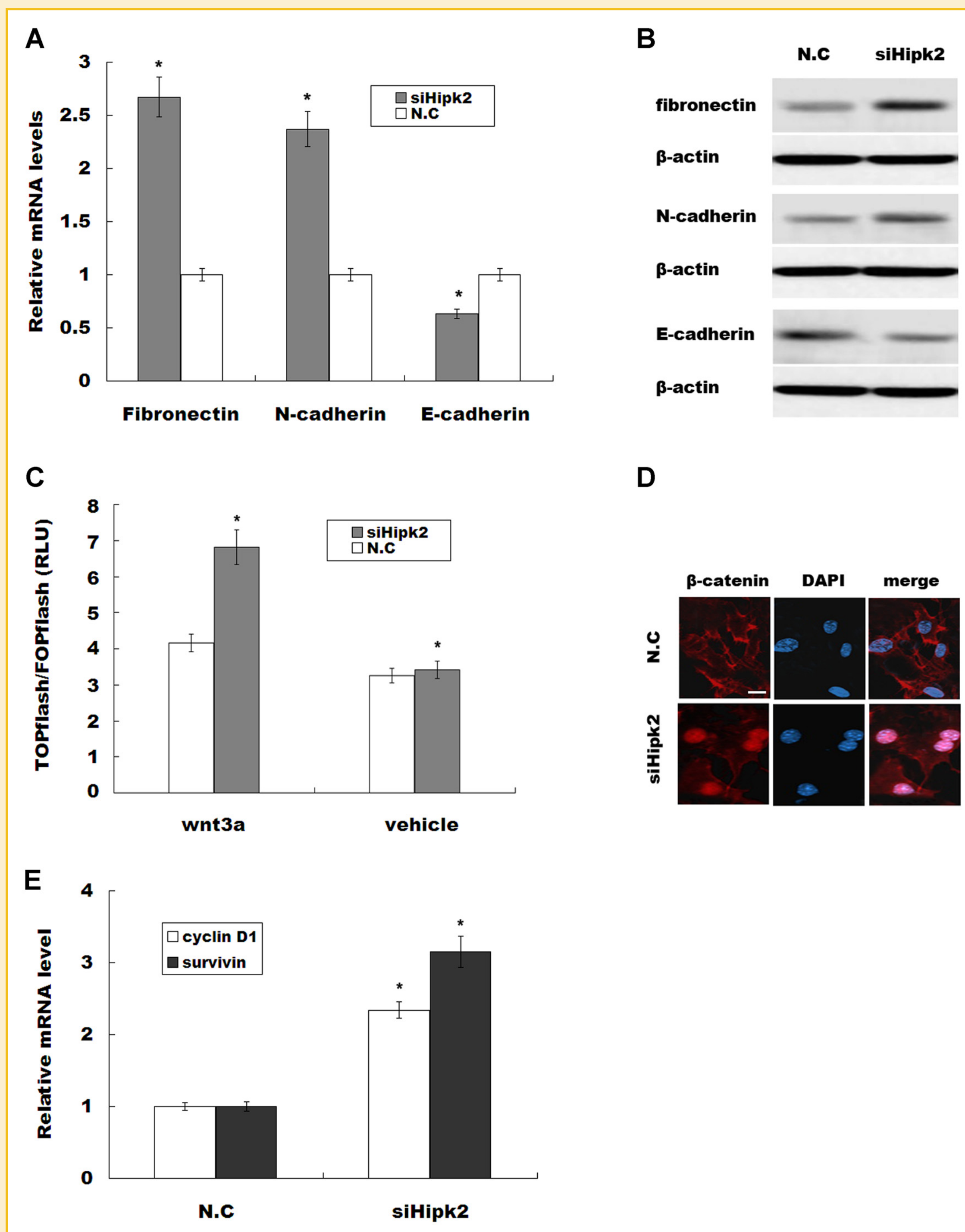

Fig. 3. Hipk2 knockdown induced an EMT phenotype in T24 cells. A,B: Measurements used quantitative RT-PCR or Western blot of the epithelial and mesenchymal markers E-cadherin, N-cadherin, and fibronectin in Hipk2-siRNA-treated T24 cells. C: Luciferase activity of TOPFlash/FOPFlash in T24 cells that were transiently transfected with Hipk2-siRNA or negative control (N.C). Data are expressed as mean ± standard deviation from five separate experiments. $^{*}P < 0.05$. D: Immunostaining analysis of β-catenin translocation in T24 cells treated with Hipk2-siRNA. Profiles are representative of at least three independent experiments. Bar = 30 μm. E: The expression levels of Wnt signaling target genes (cyclin D1 and survivin) were significantly increased in T24 cells treated with Hipk2-siRNA. $^{*}P < 0.05$.

These data suggest that Hipk2 negatively regulates bladder cancer cell invasion.

Hipk2 SILENCING ACTIVATES Wnt/β-CATENIN SIGNALING AND INDUCES EMT

We then investigated the mechanism of action for Hipk2 on the regulation of bladder cancer cell invasion. Hipk2 knockdown increased the mesenchymal markers (N-cadherin and fibronectin) mRNA levels (Fig. 3A). Meanwhile, Hipk2 knockdown decreased E-cadherin mRNA level (Fig. 3A). Moreover, E-cadherin protein level is reduced, and N-cadherin and fibronectin protein level is increased after Hipk2 inhibition (Fig. 3B), indicating that EMT is induced by downregulation of Hipk2.

The Wnt/β-catenin signaling has a particularly tight link with EMT. Nuclear translocation of β-catenin can activate the expression of Slug, which triggers an EMT and a proinvasive gene expression profile [Yook et al., 2006; Jiang et al., 2007; Ying et al., 2012]. Therefore, we investigated whether Hipk2 regulates the Wnt/β-catenin signaling. T24 cells were cotransfected with either the Wnt signaling reporter TOPFlash or the negative control FOPFlash along with either the Hipk2-siRNA or the negative control. The transfected cells were then treated with Wnt3a. Figure 3C shows that the Hipk2 knockdown significantly increased TOPFlash activity. Immunofluorescence analysis also showed that Hipk2 knockdown significantly enhanced nuclear accumulation of β-catenin after Wnt3a treatment (Fig. 3D). Consistent with these observations, the endogenous levels of Wnt target genes (cyclin D1 and survivin) were increased after Hipk2 inhibition (Fig. 3E). These data confirm that Hipk2 downregulation induces EMT, at least in part by activating the Wnt/β-catenin signaling.

Hipk2 CONTROLS BLADDER CANCER CELL INVASION BY REGULATING Wnt SIGNALING

Then we investigated whether Hipk2 regulates bladder cancer cell invasion by the Wnt signaling pathway. We first demonstrated that Hipk2 overexpression markedly inhibited bladder cancer cell invasion (Fig. 4A). Importantly, inhibition of Wnt signaling by its specific inhibitor (IWR-1) abrogated cell invasion induced by Hipk2 knockdown, though Hipk2 knockdown enhanced cell invasion (Fig. 4B). These data demonstrated that Hipk2 suppresses bladder cancer cell invasion, at least in part, by regulating Wnt/β-catenin signaling.

EMT is a key step toward cancer metastasis, and the Wnt signaling is associated with EMT [Jiang et al., 2007]. The canonical Wnt signaling is activated upon the binding of Wnt ligands to Frizzled (Fzd)/LDL-related protein (LRP) coreceptor complexes, which leads to activation of Fzd and nuclear translocation of β-catenin. β-catenin can activate the expression of Slug, which induces EMT [Eger et al., 2000]. Ying et al. [2012] showed that upregulated MALAT-1 contributes to bladder cancer cell migration by inducing EMT. MALAT-1 knockdown inhibits Wnt activation and decreases nuclear accumulation of β-catenin. They further demonstrated that MALAT-1 knockdown results in a decreased of the EMT-associated ZEB1, ZEB2, and Slug levels, and an increase of E-cadherin levels by activating Wnt signaling [Ying et al., 2012].

Hipk2 could act as a regulator of the Wnt/β-catenin signaling. Lee et al. [2009] demonstrated that Hipk2 promotes Wnt/Wingless (Wg) signaling through stabilization of β-catenin/Arm and stimulation of target gene expression. However, another study showed that Hipk2 acts as a negative regulator of the Wnt/β-catenin signaling. Inhibition of endogenous Hipk2 increases the stability of β-catenin

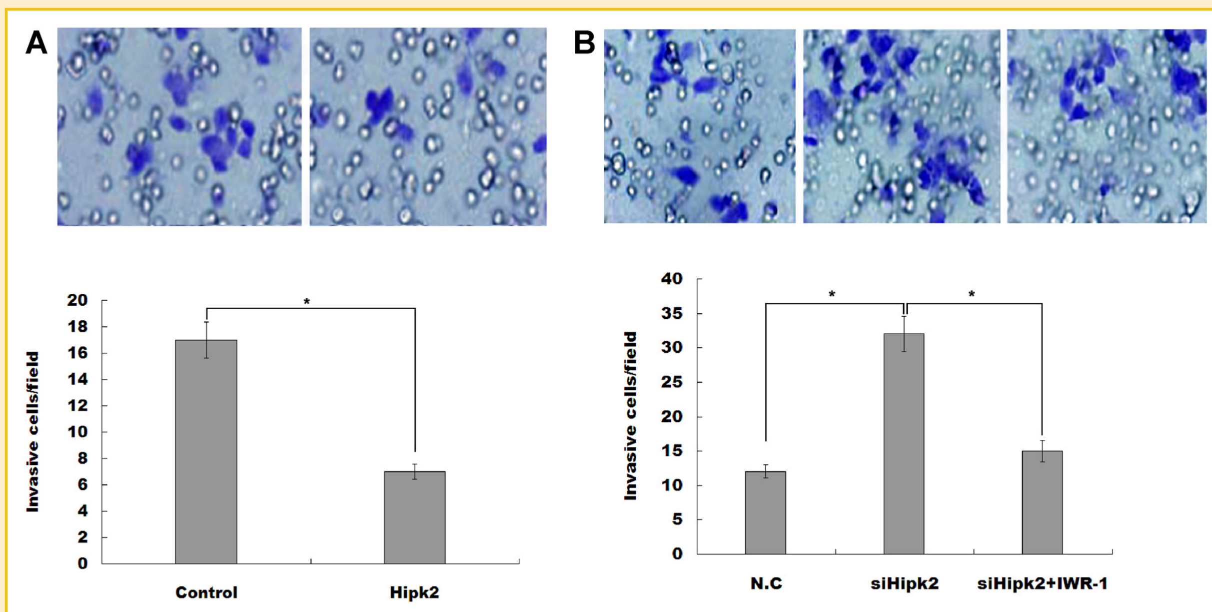

Fig. 4. Downregulated Hipk2 contributes to bladder cancer cell invasion by regulating Wnt signaling. A: Hipk2 overexpression significantly decreased bladder cancer cell invasion. Profiles, which are shown at the top, are representative of at least three independent experiments. Statistical analysis is shown at the bottom. $^{*}P < 0.05$. B: inhibition of Wnt signaling by its specific inhibitor (IWR-1) abrogated cell invasion induced by Hipk2 knockdown, though Hipk2 knockdown enhanced cell invasion. Profiles, which are shown at the top, are representative of at least three independent experiments. Statistical analysis is shown at the bottom. $^{*}P < 0.05$.

and results in the accumulation of β-catenin in the nucleus, consequently enhancing the expression of Wnt target genes and cell proliferation [Kim et al., 2010]. In the present study, we found that Hipk2 knockdown induces β-catenin nuclear localization and Wnt signaling activation. Furthermore, Hipk2 knockdown results in decrease of E-cadherin expression and increase of N-cadherin and fibronectin expression, indicated that EMT was induced. We finally demonstrated that Hipk2 inhibition promoted EMT and subsequent cell invasion, at least in part by activating Wnt signaling.

CONCLUSION

These data suggest an important role of Hipk2 in regulating metastasis of bladder cancer and implicate the potential application of Hipk2 in bladder cancer therapy.

ACKNOWLEDGEMENT

This work was supported by Shanghai Municipal Nature Science Fund (No. 13ZR1433100).

REFERENCES

Anson M, Crain-Denoyelle AM, Baud V, Chereau F, Gougelet A, Terris B, Yamagoe S, Colnot S, Viguier M, Perret C, Couty JP. 2012. Oncogenic beta-catenin triggers an inflammatory response that determines the aggressiveness of hepatocellular carcinoma in mice. J Clin Invest 122:586–599.

Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, Wu J, Li M. 2013. MicroRNA-374a activates Wnt/beta-catenin signaling to promote breast cancer metastasis. J Clin Invest 123:566–579.

Chua HL, Bhat-Nakshatri P, Clare SE, Morimiya A, Badve S, Nakshatri H. 2007. NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. Oncogene 26:711–724.

Connor KM, Hempel N, Nelson KK, Dabiri G, Gamarra A, Belarmino J, Van De Water L, Mian BM, Melendez JA. 2007. Manganese superoxide dismutase enhances the invasive and migratory activity of tumor cells. Cancer Res 67:10260–10267.

Eger A, Stockinger A, Schaffhauser B, Beug H, Foisner R. 2000. Epithelial mesenchymal transition by c-Fos estrogen receptor activation involves nuclear translocation of beta-catenin and upregulation of beta-catenin/lymphoid enhancer binding factor-1 transcriptional activity. J Cell Biol 148:173–188.

Jiang YG, Luo Y, He DL, Li X, Zhang LL, Peng T, Li MC, Lin YH. 2007. Role of Wnt/beta-catenin signaling pathway in epithelial-mesenchymal transition of human prostate cancer induced by hypoxia-inducible factor-1alpha. Int J Urol 14:1034–1039.

Kapinas K, Kessler CB, Ricks T, Gronowicz G, Delany AM. 2007. MiR-29 modulates WNT signaling in human osteoblasts through a positive feedback loop. J Biol Chem 285:25221–25231.

Kim EA, Kim JE, Sung KS, Choi DW, Lee BJ, Choi CY. 2010. Homeodomain-interacting protein kinase 2 (HIPK2) targets beta-catenin for phosphorylation and proteasomal degradation. Biochem Biophys Res Commun 394:966–971.

Lacey JV , Jr. , Devesa SS, Brinton LA. 2002. Recent trends in breast cancer incidence and mortality. Environ Mol Mutagen 39:82–88.

Lee W, Swarup S, Chen J, Ishitani T, Verheyen EM. 2009. Homeodomain-interacting protein kinases (Hipks) promote Wnt/Wg signaling through stabilization of beta-catenin/Arm and stimulation of target gene expression. Development 136:241–251.

Liu Z, Wang W, Jiang J, Bao E, Xu D, Zeng Y, Tao L, Qiu J. 2013. Downregulation of GAS5 promotes bladder cancer cell proliferation, partly by regulating CDK6. PLoS ONE 8:e73991.

Nodale C, Sheffer M, Jacob-Hirsch J, Folgiero V, Falcioni R, Aiello A, Garufi A, Rechavi G, Givol D, D'Orazi G. 2012. HIPK2 downregulates vimentin and inhibits breast cancer cell invasion. Cancer Biol Ther 13:198–205.

Pierantoni GM, Esposito F, Tornincasa M, Rinaldo C, Viglietto G, Soddu S, Fusco A. 2011. Homeodomain-interacting protein kinase-2 stabilizes p27 (kip1) by its phosphorylation at serine 10 and contributes to cell motility. J Biol Chem 286:29005–29013.

Puca R, Nardinocchi L, Pistritto G, D'Orazi G. 2008. Overexpression of HIPK2 circumvents the blockade of apoptosis in chemoresistant ovarian cancer cells. Gynecol Oncol 109:403–410.

Said N, Sanchez-Carbayo M, Smith SC, Theodorescu D. 2012. RhoGDI2 suppresses lung metastasis in mice by reducing tumor versican expression and macrophage infiltration. J Clin Invest 122:1503–1518.

Seiler R, von Gunten M, Thalmann GN, Fleischmann A. 2012. Pelvic lymph nodes: Distribution and nodal tumour burden of urothelial bladder cancer. J Clin Pathol 63:504–507.

Si W, Kang Q, Luu HH, Park JK, Luo Q, Song WX, Jiang W, Luo X, Li X, Yin H, Montag AG, Haydon RC, He TC. 2006. CCN1/Cyr61 is regulated by the canonical Wnt signal and plays an important role in Wnt3A-induced osteoblast differentiation of mesenchymal stem cells. Mol Cell Biol 26:2955–2964.

Tano K, Mizuno R, Okada T, Rakwal R, Shibato J, Masuo Y, Ijiri K, Akimitsu N. 2010. MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. FEBS Lett 584:4575–4580.

Wei G, Ku S, Ma GK, Saito S, Tang AA, Zhang J, Mao JH, Appella E, Balmain A, Huang EJ. 2007. HIPK2 represses beta-catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis. Proc Natl Acad Sci USA 104:13040–13045.

Wu ZQ, Brabletz T, Fearon E, Willis AL, Hu CY, Li XY, Weiss SJ. 2012. Canonical Wnt suppressor, Axin2, promotes colon carcinoma oncogenic activity. Proc Natl Acad Sci USA 109:11312–11317.

Ying L, Chen Q, Wang Y, Zhou Z, Huang Y, Qiu F. 2012. Upregulated MALAT-1 contributes to bladder cancer cell migration by inducing epithelial-to-mesenchymal transition. Mol Biosyst 8:2289–2294.

Yook JI, Li XY, Ota I, Hu C, Kim HS, Kim NH, Cha SY, Ryu JK, Choi YJ, Kim J, Fearon ER, Weiss SJ. 2006. A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells. Nat Cell Biol 8:1398–1406.

Zhang Q, Nottke A, Goodman RH. 2005. Homeodomain-interacting protein kinase-2 mediates CtBP phosphorylation and degradation in UV-triggered apoptosis. Proc Natl Acad Sci USA 102:2802–2807.