Biochemical and Biophysical Research Communications 394 (2010) 966–971

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Homeodomain-interacting protein kinase 2 (HIPK2) targets β-catenin for phosphorylation and proteasomal degradation

Eun-A Kim [a], Ji Eon Kim [b], Ki Sa Sung [a], Dong Wook Choi [a], Byeong Jae Lee [b], Cheol Yong Choi [a,*]

[a] Department of Biological Science, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[b] School of Biological Sciences and Institute of Molecular Biology and Genetics, Seoul National University, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 12 March 2010
Available online 20 March 2010

Keywords:
HIPK2
β-Catenin phosphorylation
Proteasomal degradation

ABSTRACT

The regulation of intracellular β-catenin levels is central in the Wnt/β-catenin signaling cascade and the activation of the Wnt target genes. Here, we show that homeodomain-interacting protein kinase 2 (HIPK2) acts as a negative regulator of the Wnt/β-catenin pathway. Knock-down of endogenous HIPK2 increases the stability of β-catenin and results in the accumulation of β-catenin in the nucleus, consequently enhancing the expression of Wnt target genes and cell proliferation both *in vivo* and in cultured cells. HIPK2 inhibits TCF/LEF-mediated target gene activation via degradation of β-catenin. HIPK2 phosphorylates β-catenin at its Ser33 and Ser37 residues without the aid of a priming kinase. Substitutions of Ser33 and Ser37 for alanines abolished the degradation of β-catenin associated with HIPK2. In *ex vivo* mouse model, HIPK2 knock-down resulted in accumulation of β-catenin, thereby potentiated β-catenin-mediated cell proliferation and tumor formation. Furthermore, the axis duplication induced by the ectopic expression of β-catenin was blocked by co-injection of HIPK2 mRNAs into *Xenopus* embryos. Taken together, HIPK2 appears to function as a novel negative regulator of β-catenin through its phosphorylation and proteasomal degradation.

1. Introduction

The Wnt/β-catenin signaling pathway plays important roles in animal development [1]. The aberrant regulation of Wnt signaling cascade component is associated with human disease, and mutations of APC, Axin and β-catenin have been studied extensively in colon and liver cancers [2]. Although many signaling molecules participate in the transduction of signals from the Wnt receptor to downstream components, β-catenin plays a pivotal role in the signaling pathway. In the absence of the Wnt signal, cytosolic β-catenin is constitutively degraded via phosphorylation-dependent ubiquitination and subsequent proteasomal clearance. The Wnt ligand/receptor-induced signaling cascade results in stabilization of β-catenin and an increase in the levels of β-catenin in the nucleus where it functions as a coregulator of TCF/LEF transcription factors for Wnt target gene activation [3,4]. The canonical mechanism of β-catenin regulation involves a destruction complex where β-catenin is phosphorylated by priming kinases at the Ser45 site and subsequently by glycogen synthase kinase 3β (GSK3β) at the Thr41, Ser37 and Ser33 sites [5,6]. Phosphorylated β-catenin is recognized by β-TrCP, a component of the SCF E3 ligase complex, and is degraded by the ubiquitin-mediated proteasome system [7,8].

HIPK2 has been shown to act as a coregulator of homeodomain transcription factors [9–12] and as a tumor suppressor through the phosphorylation of cellular target proteins, including p53, CtBP, AML and p300/CBP [13,14]. HIPK2 appears to exert multiple functions depending on the binding partner or on the phosphorylation of the downstream target proteins in different signaling pathways. Although there is evidence supporting that HIPK2 may be involved in the Wnt signaling pathway [15,16,22], it was not well known how HIPK2 directly regulates intracellular β-catenin levels. In this study, we report that HIPK2 can bind directly and phosphorylate β-catenin, and consequently degrades β-catenin. The knock-down of endogenous HIPK2 augments the stability of β-catenin and the expression of β-catenin target genes. A stable tumor cell line in which HIPK2 was silenced using an HIPK2 shRNA displays accelerated proliferation. In addition, HIPK2 expression blocked the axis duplication induced by the injection of β-catenin mRNA into *Xenopus* embryos. These results strongly indicate that HIPK2 is a novel negative regulator of Wnt signaling operating via the direct phosphorylation and degradation of β-catenin, a key component of the Wnt signaling pathway.

* Corresponding author. Fax: +82 31 290 7015.
E-mail address: choicy@skku.ac.kr (C.Y. Choi).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.03.099

2. Materials and methods

2.1. Cell transfection and luciferase reporter assays

Cell cultures, transient transfection and luciferase assays were performed as described previously [17,18]. The HEK293 and RKO cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco). Cells were grown on six-well plates and transiently transfected with reporter plasmids and various expression plasmids using Fugene6 reagent (Roche Molecular Biochemicals). For the transcription assays, cells were transfected with 0.4 μg of TOP-FLASH reporter plasmids, 0.6 μg of β-catenin plasmid, 2 μg of HIPK2/K221R plasmids and 0.1 μg of pCMV-β-gal plasmid encoding β-galactosidase was included in all transfections to normalize the transfection efficiency in combination as indicated in figures. Thirty-six hours after transfection, the luciferase activities were measured from duplicate plates using the Luciferase Reporter Assay System (Promega) and a Genios luminometer (TECAN). All experiments were repeated at least three times.

2.2. In vitro phosphorylation of β-catenin

In vitro β-catenin phosphorylation was performed as described previously [9]. In brief, affinity-purified GST-β-catenin was mixed with GST-HIPK2 (aa 1–629) in 30 μl of kinase buffer (50 mM HEPES pH 7.0, 0.1 mM EDTA, 0.1% β-mercaptoethanol, 0.1 mg/ml bovine serum albumin, 0.15 M NaCl, 5 mM $MgCl_2$) and incubated for 30 min at 30 °C. The samples were resolved by SDS–PAGE, and the amounts of phosphorylation were evaluated by Western blotting with Ser33, Ser37-specific anti-phospho-β-catenin antibody (Cell signaling).

See Materials and methods section of Supplementary data for detailed experimental procedures for plasmid constructions, mRNA injection into *Xenopus* embryo, reverse transcription-PCR, GST pull-down assays, Western blot and immunoprecipitation, Establishment of shRNA-expressing cell lines and subcutaneous injection of CT-26 cells into BALB/c mice.

3. Results

3.1. Knock-down of HIPK2 increases β-catenin stability

In a yeast-two hybrid screen using the C-terminus (aa 503–1189) of HIPK2 as bait, we identified β-catenin as a HIPK2-interacting protein. The association of endogenous HIPK2 with β-catenin was also verified via co-immunoprecipitation with anti-β-catenin antibody followed by Western blotting using anti-HIPK2 antibody (Fig. S1). In the course of co-immunoprecipitation experiments, we have seen that the level of β-catenin was decreased by forced expression of HIPK2. Therefore, we assessed the effects of HIPK2 silencing on the level of β-catenin and the β-catenin-mediated transcriptional activation. The knock-down of endogenous HIPK2 enhanced the level of β-catenin (Fig. 1A) and β-catenin-mediated transactivation of the *cyclin D* promoter as well as the TOP-FLASH reporter gene (Fig. 1B and C). As a positive control, the expression plasmid for shRNA against GSK3β was utilized in parallel. Since β-catenin is expressed at a relatively low level and is not associated with plasma membrane in RKO cells [19], RKO cell was chosen for establishment of HIPK2 knock-down cell line to study the effect of HIPK2 on β-catenin stability. The stability of β-catenin was remarkably enhanced in RKO-shHIPK2 cells when compared with that observed in RKO cells (Fig. 1D). These results indicate that the downregulation of HIPK2 increases the stability of β-catenin. We further evaluated the effects of HIPK2 silencing on cellular functions of β-catenin in terms of its localization and activation of target genes. The immunocytochemistry of endogenous β-catenin showed that β-catenin in the RKO-shHIPK2 cell line was detected in both the nucleus and the cytoplasm, while β-catenin in RKO cells was detected in the cytoplasm. Additionally, the quantity of β-catenin, which was determined by intensities of fluorescence, was increased to a greater degree in RKO-shHIPK2 cells than in RKO cells (Fig. 1E). Consistently, the expressions of *c-myc*, *cyclin D* and *DKK1*, typical target genes of β-catenin, were increased to a higher degree in RKO-shHIPK2 cells than in RKO cells (Fig. 1F and G). Taken together, HIPK2 knock-down resulted in the stabilization and concomitant translocalization of β-catenin to the nucleus, and transactivation of β-catenin target genes.

3.2. HIPK2 down-regulates Wnt signaling-mediated transactivation through β-catenin degradation

In order to characterize function of HIPK2 on the regulation of β-catenin, a series of experiments was carried out using transient expression of wild-type and catalytically inactive HIPK2 mutant. Transcription assays were conducted via the transfection of Wnt3a or β-catenin expression plasmids in combination with wild-type or catalytically inactive HIPK2. As shown in Fig. 2A, HIPK2 repressed the Wnt3a- and Wnt1-mediated transactivation of reporter gene. HIPK2 also inhibited β-catenin-mediated transactivation of the reporter gene in a catalytic activity-dependent manner (Fig. 2B). In addition, transcription from the promoter of Wnt target gene, such as *cyclin D*, was repressed by HIPK2 (Fig. 2C). These results indicate that HIPK2 inhibits Wnt/β-catenin-mediated transactivation of target genes.

Because HIPK2 knock-down increased β-catenin stability, we wondered whether β-catenin is degraded by HIPK2. The Western blot revealed that HIPK2 remarkably reduced β-catenin stability, which was blocked by administration of cells with MG132, thereby indicating that HIPK2 induced the proteasomal degradation of β-catenin as GSK3β (Fig. 2D). Consistently, β-catenin stability was dramatically reduced upon HIPK2 expression (Fig. 2E, HIPK2 panel). The inhibitory effect of HIPK2 was compromised by the inactivation of its catalytic activity (Fig. 2E, HIPK2 KR panel). The expression of the HIPK2 N-terminus with an active catalytic domain (HIPK2 1–629) resulted in suppression of β-catenin-mediated transactivation (Fig. 2B, lane 5) and β-catenin degradation (Fig. 2F), in contrast to the catalytically inactive mutant HIPK2 (HIPK2 1–629 K221R) (Fig. 2B and F). Taken together, these results suggest that HIPK2 represses transactivation of Wnt target genes through β-catenin degradation.

3.3. HIPK2 targets β-catenin for phosphorylation and proteasomal degradation

Phosphorylation-dependent β-catenin regulation by HIPK2 led us to test whether HIPK2 can directly phosphorylate β-catenin. The Western blot analysis revealed that the level of β-cat ΔN was not influenced by HIPK2 expression while wild-type β-catenin was decreased by HIPK2 in a manner depending on catalytic activity of HIPK2 (Fig. 3A). These results indicate that degradation of β-catenin by HIPK2 depend on the N-terminal phosphorylation sites of β-catenin in a HIPK2's catalytic activity-dependent manner, suggesting that N-terminal region of β-catenin might be a phosphorylation target of HIPK2. In cultured HEK293 cells, the coexpression of HIPK2 and β-catenin resulted in β-catenin phosphorylation, which was determined by Western blot using the S33- and S37-specific phospho-β-catenin antibody (Fig. 3B). The affinity-purified wild-type β-catenin was phosphorylated by HIPK2 *in vitro*, but substitutions of Ser33 and Ser37 to alanines abolished its phosphory-

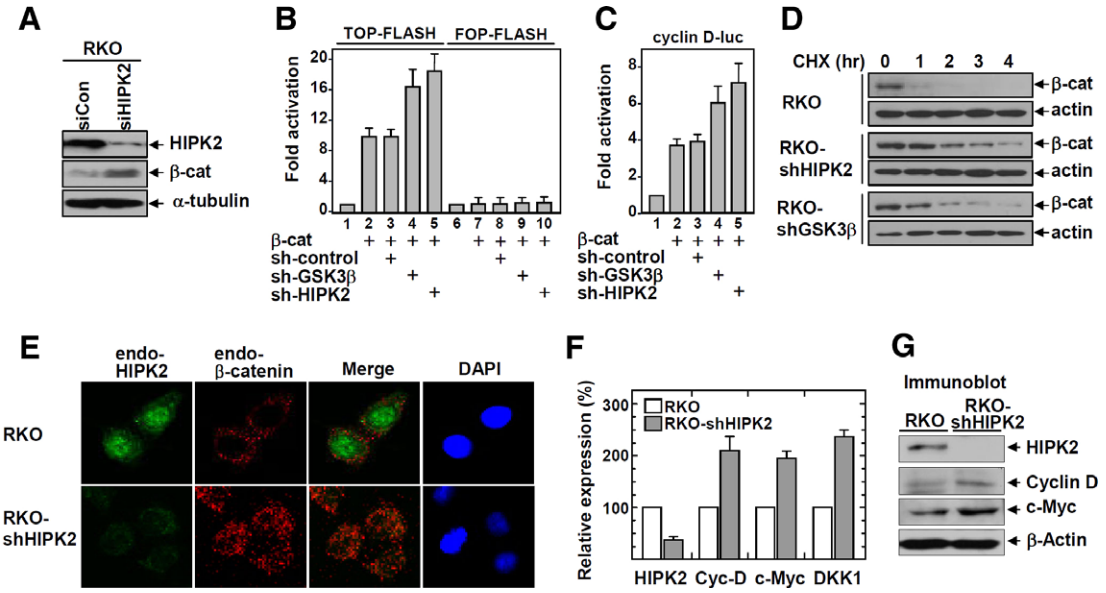

Fig. 1. Knock-down of endogenous HIPK2 increases stability and transcription activity of β-catenin. (A) RKO cells were transfected with either control siRNA or HIPK2 siRNA, and the levels of endogenous HIPK2 and β-catenin were determined by Western blotting using anti-HIPK2 and anti-β-catenin antibodies. (B and C) TOP-FLASH, FOP-FLASH or cyclin D-luciferase reporter plasmids were transfected into HEK293 cells together with expression plasmids for β-catenin and shRNA against either the scrambled sequence (as a control), GSK3β or HIPK2. Thirty-six hours after transfection, normalized luciferase activities, obtained from transfected cells with indicated expression plasmids, was divided by the corresponding value obtained with the empty vector and is shown as fold activation. (D) Each of RKO cells (RKO), RKO cells silencing HIPK2 (RKO-shHIPK2) and RKO cells silencing GSK3β (RKO-shGSK3β) were treated with cycloheximide for the time indicated in figure. Endogenous levels of β-catenin were determined to measure the stability of β-catenin by Western blotting using anti-β-catenin antibody. (E) Cells were stained with anti-β-catenin or anti-HIPK2 antibodies, and localizations of endogenous β-catenin and HIPK2 in either RKO or RKO-shHIPK2 cell line were analyzed by confocal microscopy. Note that, in RKO-shHIPK2 cells, β-catenin is localized to the nucleus. Cells were also stained with DAPI to show the nucleus. (F) Knock-down of HIPK2 enhances expression of endogenous Wnt/β-catenin target genes. mRNAs were isolated from either RKO or RKO-shHIPK2 cells, followed by qRT-PCR using either primers for cyclin D, c-myc, DKK1, or HIPK2. (G) Total cell lysates were prepared from either RKO or RKO-shHIPK2 cells, and were subjected to Western blotting with either anti-HIPK2, anti-cyclin D, or anti-c-myc antibody.

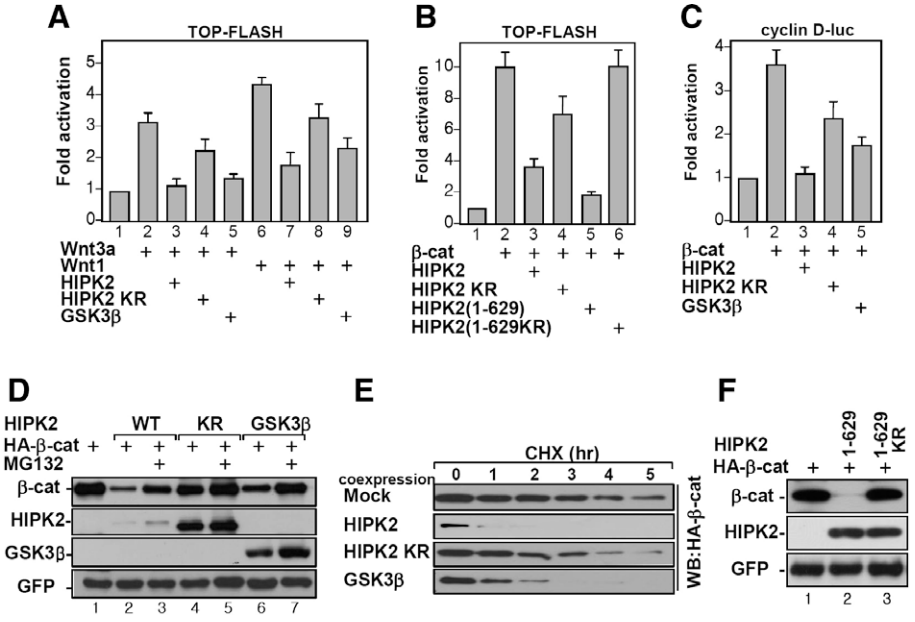

Fig. 2. HIPK2 down-regulates Wnt signaling-mediated transactivation via β-catenin degradation. (A and B) HEK293 cells were transfected with TOP-FLASH reporter plasmid together with expression plasmids for Wnt1, Wnt3a, β-catenin, HIPK2, HIPK2(K221R), HIPK2(1–629), HIPK2(1–629KR) and GSK3β as indicated in the figures. Thirty-six hours after transfection, normalized luciferase activities, obtained from transfected cells with indicated expression plasmids, was divided by the corresponding value obtained with the empty vector and is shown as fold activation. (C) Cyclin D-luciferase reporter plasmid was transfected into HEK293 cells, together with β-catenin expression plasmids in combination with either HIPK2, HIPK2(K221R) or GSK3β expression plasmid. (D) HA-β-catenin expression plasmid was transfected into HEK293 cells together with either HIPK2, HIPK2(K221R) or GSK3β expression plasmid. GFP expression plasmids were transfected into the cell to monitor the relative transfection efficiency. Thirty-six hours after transfection, half of the transfected cell were treated with the MG132, proteasome inhibitor for 10 h, followed by Western blot analysis for the detection of HA-β-catenin. (E) HA-β-catenin expression plasmids were transfected into HEK293 cells together with either HIPK2, HIPK2(K221R) or GSK3β expression plasmids. Thirty-six hours after transfection, cells were chased with cycloheximide (CHX) (50 μg/ml) to inhibit *de novo* protein synthesis. At the indicated times after the addition of cycloheximide, the levels of the β-catenin proteins were analyzed by Western blot analysis. (F) HA-β-catenin expression plasmid was transfected into HEK293 cells together with either HIPK2(1–629) or HIPK2(1–629 K221R). Thirty-six hours after transfection, the level of HA-β-catenin was determined with Western blotting using anti-HA antibody.

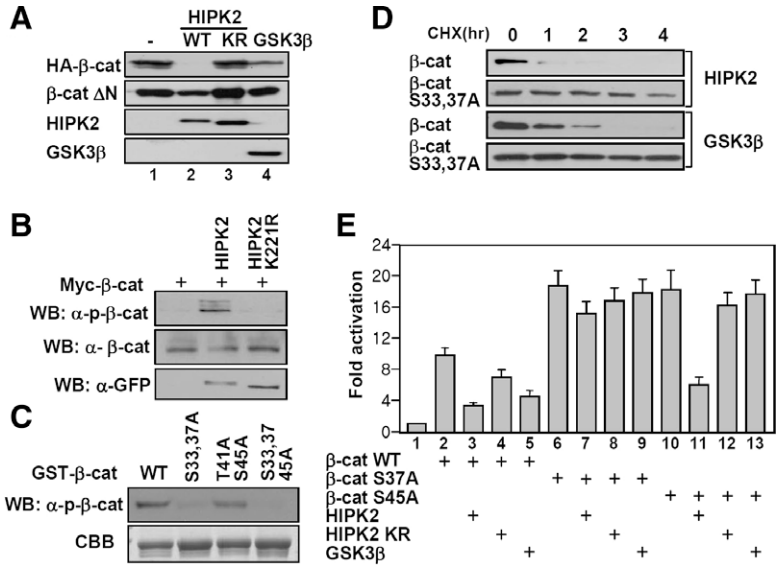

Fig. 3. Phosphorylation and degradation of β-catenin by HIPK2. (A) Either HA-β-catenin or HA-β-catenin ΔN (deletion of amino acid 1–89) expression plasmid was transfected into HEK293 cells together with expression plasmid for either HIPK2 (WT), HIPK2(K221R) (KR) or GSK3β. Levels of wild-type or mutant β-catenin were analyzed by Western blot with anti-HA antibody. (B) HEK293 cells expressing Myc-β-catenin and either GFP-HIPK2 or GFP-HIPK2(K221R) were treated with MG132 for 12 h and then β-catenin was immunoprecipitated from the cell lysates. Phosphorylation of β-catenin at Ser33 and Ser37 sites in HEK293 cells were determined by Western blot with phosphorylated S33- and S37-specific anti-β-catenin antibody. (C) Affinity-purified wild-type and GST-β-catenin mutants were subjected to *in vitro* phosphorylation reaction using affinity-purified GST-HIPK2. The identical amounts (2 μg) of GST-β-catenin and mutants of GST-β-catenin were incubated with GST-HIPK2, and phosphorylated β-catenin was detected with phosphorylated S33- and S37-specific anti-β-catenin antibody. GST-β-catenin was stained with Coomassie Brilliant Blue (CBB). (D) Either HA-β-catenin or HA-β-catenin S33, 37A mutant was expressed in combination with HIPK2 or GSK3β, and transfected cells were administered with cycloheximide, and levels of β-catenin were determined by Western blot. (E) Three different expression plasmids for β-catenin (wild-type, lanes 2–5; S37A mutant, lanes 6–9; S45A mutant, lanes 10–13) were transfected into HEK293 cells in combination with either HIPK2, HIPK2(K221R) (KR) or GSK3β expression plasmid. Transcription activities from the TOP-FLASH reporter plasmid are shown.

lation. In addition, substitutions of Thr41 and Ser45 to alanines did not affect its phosphorylation by HIPK2, indicating that phosphorylation of β-catenin by HIPK2 does not require priming kinase which phosphorylate Ser45 for following phosphorylation of Thr41, Ser37 and Ser33 by GSK3β (Fig. 3C). Moreover, chase experiments after administration of cycloheximide indicated that the β-catenin S33, 37A mutant was almost resistant to degradation upon HIPK2 expression (Fig. 3D). These results were supported by the observation that the transactivation of the reporter gene by β-cat S37A was not affected as the result of HIPK2 expression, whereas transactivations by wild-type β-catenin and β-cat S45A were inhibited by HIPK2 (Fig. 3E). These results suggest that HIPK2 phosphorylate S33 and S37 residues of β-catenin *in vitro* and *in vivo*, and the phosphorylation of β-catenin by HIPK2 may contribute to the degradation of β-catenin.

3.4. Knock-down of HIPK2 increases β-catenin-mediated cell proliferation both in vivo and in cultured cells

Next, we wished to determine the *in vivo* significance of these observations in cellular level and *ex vivo* grafting mouse model. As β-catenin has oncogenic potential through the promotion of the cell proliferation in RKO cells [20], proliferation and migration of RKO-shHIPK2 cells were monitored by measuring the time required for the closure of scratch-wounded gaps. Wound closure was completed more quickly in the RKO-shHIPK2 cells than in the RKO cells (Fig. 4A, left panel). The accelerated cell proliferation and migration of the RKO-shHIPK2 cells was reversed via the expression of the dominant negative Tcf3 mutant [21] and constitutive repressor form of TCf3 (Fig. 4A, right panel and B), thereby indicating that enhanced proliferation potential is the result of the β-catenin accumulation induced by HIPK2 silencing. *Ex vivo* grafting of tumor cells is one of the experimental approaches utilized to explore the effect of a gene on tumor cell growth in a murine model. The mouse colon cancer line (CT-26) expressing shHIPK2 (CT-26-shHIPK2) was established and subcutaneously injected into the dorsal region of the syngenic BALB/c mice and tumor outgrowth was monitored for 2 weeks. The subcutaneous injection of CT-26-shHIPK2 cells resulted in enhanced tumor outgrowth as compared with that observed in the control CT-26 cells. The average weight of the tumors ($n = 10$) derived from injection of the CT-26-shHIPK2 cells was fourfold higher than that of the tumors ($n = 8$) derived from injection of the control CT-26 cells (Fig. 4C). The Western blot analysis indicated that β-catenin protein levels from the CT-26-shHIPK2 tumors were higher than the β-catenin protein levels from the CT-26 tumors (Fig. 4D). Taken together, the knock-down of HIPK2 expression via shRNA enhanced the stability of β-catenin and target gene expression. Consequently, the growth of tumor cells was accelerated *in vivo* as the result of β-catenin accumulation. Furthermore, we determined whether HIPK2 is involved in the Wnt/β-catenin pathway in *Xenopus* embryo (Fig. S2). The injection of *in vitro* transcribed HIPK2 mRNA together with β-catenin mRNA blocked the axis duplication induced by the forced expression of β-catenin (Fig. S2B). In accordance with the phenotypes of the *Xenopus* embryos, the expressions of all tested Wnt target genes (*Siamois*, *Xnr3*, *xCyclin D* and *Gsc*) were attenuated by coexpression of HIPK2 (Fig. S2C). These results indicate that HIPK2 is a negative regulator of the Wnt/β-catenin signaling pathway *in vivo*.

4. Discussion

In this study, we have demonstrated that HIPK2 is a negative regulator of the Wnt/β-catenin signaling pathway through the direct phosphorylation and degradation of β-catenin. Recent papers associating HIPK2 with the Wnt/β-catenin signaling pathway have

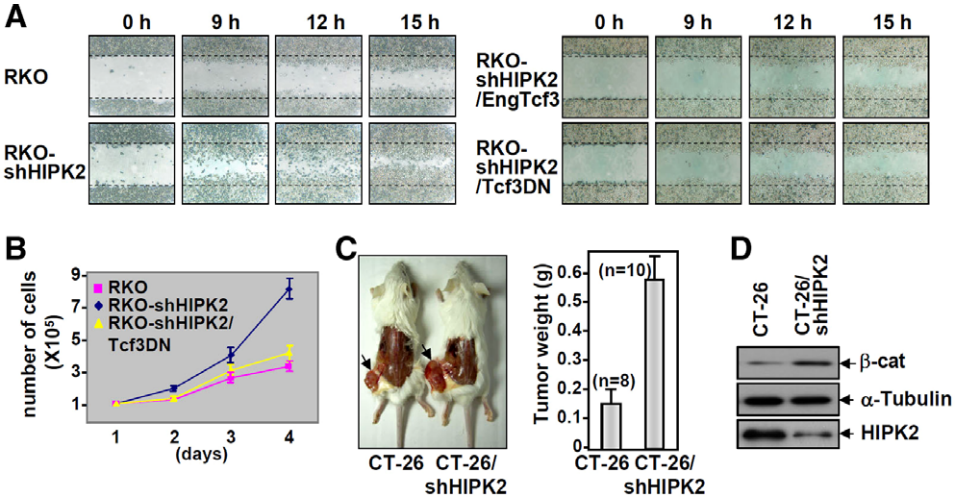

Fig. 4. Knock-down of endogenous HIPK2 promotes cell proliferation through β-catenin accumulation. (A) Either RKO, RKO-shHIPK2, RKO-HIPK2/TcfDN (expressing transiently dominant negative Tcf3) or RKO-shHIPK2/EngTcf3 (expressing transiently constitutive repressor form of Tcf3) cells were grown to confluence under normal culture conditions and were scratched out to make a gap. The closures of gaps after scratching at the indicated time points are shown. (B) The numbers of cells were counted for three days after seeding the same number (1×10^5 cells) of either RKO, RKO-shHIPK2 or RKO-shHIPK2/Tcf3DN cells. (C) Either CT-26 or CT-26/shHIPK2 cells (2×10^6 cells/mouse) were injected subcutaneously into BALB/c mice. Arrows indicate tumors originated from either injected CT-26 or CT-26/shHIPK2 cells. Graph is weight presentation of tumors from either CT-26 ($n = 8$) or CT-26/shHIPK2 ($n = 10$). (D) Endogenous levels of β-catenin in tumor isolated from the mice as shown in C were determined by Western blotting with anti-HIPK2 and anti-β-catenin antibodies.

reported conflicting results regarding whether HIPK2 is involved in proteasomal degradation of β-catenin [16,22,23]. For example, Puca et al. have shown that HIPK2 downregulates β-catenin protein levels and inhibits β-catenin transcriptional activity in a catalytic activity-dependent way, and contradictory to results from other groups including ours, Wei et al. showed that the level of β-catenin was not affected in HIPK2 KO MEF cells and over-expression of HIPK2 did not induce β-catenin degradation [16,22]. These conflicting results may be due to differences between cell lines, since empirically, we have also found that certain cell lines do not show elevated levels of β-catenin upon HIPK2 knock-down (data not shown). HIPK2 may induce proteasomal degradation of β-catenin in a cell type-specific manner, requiring different degradation components such as E3 ubiquitin ligases depending on the cellular context. HIPK2-mediated degradation of β-catenin was induced in a dose- and phosphorylation-dependent manner (Figs. 2D, F and 3A). Wei et al. showed that deletion of the YH domain abolished repressor activity of HIPK2 upon β-catenin-mediated transactivation [16]. Further deletion, however, of the whole C-terminus (deletion of amino acids to 522 or 753) restored the repressor activity of HIPK2 (Eric J. Huang, personal communication). Consistent with these results, we observed that the N-terminal of HIPK2 (1–629) showed stronger repressor activity than wild-type HIPK2 (Fig. 2B, lane 5). Degradation of β-catenin by HIPK2 (1–629) was found to be catalytic activity-dependent, since the catalytically inactive HIPK2 (1–629) K221R mutant could not degrade β-catenin (Fig. 2F, lane 3). These results suggest that HIPK2 suppresses the function of β-catenin by two levels of regulation; at the transcriptional level through the HIPK2 C-terminus, and at the protein level through HIPK2 catalytic activity. HIPK1 was also shown to contribute to Dsh-dependent signaling activities in a complex fashion during early *Xenopus* development, and the signaling functions of HIPK1 were suggested to be context-dependent [24]. Although it is evident that the regulation of Wnt/β-catenin signaling by HIPKs is a complex process, much is still unknown, including the possibility of functional redundancy between HIPK1 and HIPK2 [25,26].

Given that the protein level and catalytic activity of HIPK2 could be elevated and activated, respectively, by a variety of external stimuli [13,14], it is conceivable that HIPK2 functions as a signal transducer that responds to various stimuli for the downregulation of the Wnt signaling pathway in both catalytic activity-dependent and -independent manners. Studies regarding the upstream signaling pathways for the stabilization and activation of HIPK2 could provide further insight into the HIPK2-mediated β-catenin regulation.

Acknowledgments

We would like to thank Drs. E.H. Jho, S.H. Baek and C. Choi for plasmids and cells. This work was supported by Korea Science and Engineering Foundation (KOSEF) Grant funded by the government of the Republic of Korea (Drug Discovery Research Program, M10648000092-08N4800-09210 and the Ubiquitome Research Program, 2009-00983 to C.Y.C.) and by the Korean Government (Ministry of Education, Science and Technology) (KRF-2005-005-J16001 to B.J.L.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.03.099.

References

[1] C.Y. Logan, R. Nusse, The Wnt signaling pathway in development and disease, Annu. Rev. Cell Dev. Biol. 20 (2004) 781–810.
[2] R.T. Moon, A.D. Kohn, G.V. De Ferrari, A. Kaykas, WNT and β-catenin signalling: diseases and therapies, Nat. Rev. Genet. 5 (2004) 691–701.
[3] M. Shtutman, J. Zhurinsky, I. Simcha, C. Albanese, M. D'Amico, R. Pestell, A. Ben-Ze'ev, The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway, Proc. Natl. Acad. Sci. USA 96 (1999) 5522–5527.
[4] O. Tetsu, F. McCormick, β-Catenin regulates expression of cyclin D1 in colon carcinoma cells, Nature 398 (1999) 422–426.
[5] C. Liu, Y. Li, M. Semenov, C. Han, G.H. Baeg, Y. Tan, Z. Zhang, X. Lin, X. He, Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism, Cell 108 (2002) 837–847.
[6] S. Yanagawa, Y. Matsuda, J.S. Lee, H. Matsubayashi, S. Sese, T. Kadowaki, A. Ishimoto, Casein kinase I phosphorylates the Armadillo protein and induces its degradation in *Drosophila*, EMBO J. 21 (2002) 1733–1742.
[7] E. Latres, D.S. Chiaur, M. Pagano, The human F box protein β-Trcp associates with the Cul1/Skp1 complex and regulates the stability of β-catenin, Oncogene 18 (1999) 849–854.

[8] J.T. Winston, P. Strack, P. Beer-Romero, C.Y. Chu, S.J. Elledge, J.W. Harper, The SCFβ–TRCP–ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IκBα and β-catenin and stimulates IκBα ubiquitination in vitro, Genes Dev. 13 (1999) 270–283.
[9] C.Y. Choi, Y.H. Kim, Y.O. Kim, S.J. Park, E.A. Kim, W. Riemenschneider, K. Gajewski, R.A. Schulz, Y. Kim, Phosphorylation by the DHIPK2 protein kinase modulates the corepressor activity of Groucho, J. Biol. Chem. 280 (2005) 21427–21436.
[10] C.Y. Choi, Y.H. Kim, H.J. Kwon, Y. Kim, The homeodomain protein NK-3 recruits Groucho and a histone deacetylase complex to repress transcription, J. Biol. Chem. 274 (1999) 33194–33197.
[11] Y.H. Kim, C.Y. Choi, S.J. Lee, M.A. Conti, Y. Kim, Homeodomain-interacting protein kinases, a novel family of co-repressors for homeodomain transcription factors, J. Biol. Chem. 273 (1998) 25875–25879.
[12] A.K. Wiggins, G. Wei, E. Doxakis, C. Wong, A.A. Tang, K. Zang, E.J. Luo, R.L. Neve, L.F. Reichardt, E.J. Huang, Interaction of Brn3a and HIPK2 mediates transcriptional repression of sensory neuron survival, J. Cell Biol. 167 (2004) 257–267.
[13] M.A. Calzado, F. Renner, A. Roscic, M.L. Schmitz, HIPK2: a versatile switchboard regulating the transcription machinery and cell death, Cell Cycle 6 (2007) 139–143.
[14] C. Rinaldo, A. Prodosmo, F. Siepi, S. Soddu, HIPK2: a multitalented partner for transcription factors in DNA damage response and development, Biochem. Cell Biol. 85 (2007) 411–418.
[15] C. Kanei-Ishii, J. Ninomiya-Tsuji, J. Tanikawa, T. Nomura, T. Ishitani, S. Kishida, K. Kokura, T. Kurahashi, E. Ichikawa-Iwata, Y. Kim, K. Matsumoto, S. Ishii, Wnt-1 signal induces phosphorylation and degradation of c-Myb protein via TAK1, HIPK2, and NLK, Genes Dev. 18 (2004) 816–829.
[16] G. Wei, S. Ku, G.K. Ma, S. Saito, A.A. Tang, J. Zhang, J.H. Mao, E. Appella, A. Balmain, E.J. Huang, HIPK2 represses beta-catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis, Proc. Natl. Acad. Sci. USA 104 (2007) 13040–13045.
[17] E.A. Kim, Y.T. Noh, M.J. Ryu, H.T. Kim, S.E. Lee, C.H. Kim, C. Lee, Y.H. Kim, C.Y. Choi, Phosphorylation and transactivation of Pax6 by homeodomain-interacting protein kinase 2, J. Biol. Chem. 281 (2006) 7489–74897.
[18] K.S. Sung, Y.Y. Go, J.H. Ahn, Y.H. Kim, Y. Kim, C.Y. Choi, Differential interactions of the homeodomain-interacting protein kinase 2 (HIPK2) by phosphorylation-dependent sumoylation, FEBS Lett. 579 (2005) 3001–3008.
[19] M.B. Major, N.D. Camp, J.D. Berndt, X. Yi, S.J. Goldenberg, C. Hubbert, T.L. Biechele, A.C. Gingras, N. Zheng, M.J. Maccoss, S. Angers, R.T. Moon, Wilms tumor suppressor WTX negatively regulates WNT/β-catenin signaling, Science 316 (2007) 1043–1046.
[20] R. Firestein, A.J. Bass, S.Y. Kim, I.F. Dunn, S.J. Silver, I. Guney, E. Freed, A.H. Ligon, N. Vena, S. Ogino, M.G. Chheda, P. Tamayo, S. Finn, Y. Shrestha, J.S. Boehm, S. Jain, E. Bojarski, C. Mermel, J. Barretina, J.A. Chan, J. Baselga, J. Tabernero, D.E. Root, C.S. Fuchs, M. Loda, R.A. Shivdasani, M. Meyerson, W.C. Hahn, CDK8 is a colorectal cancer oncogene that regulates β-catenin activity, Nature 455 (2008) 547–551.
[21] C.H. Kim, T. Oda, M. Itoh, D. Jiang, K.B. Artinger, S.C. Chandrasekharappa, W. Driever, A.B. Chitnis, Repressor activity of Headless/Tcf3 is essential for vertebrate head formation, Nature 407 (2000) 913–916.
[22] R. Puca, L. Nardinocchi, G. D'Orazi, Regulation of vascular endothelial growth factor expression by homeodomain-interacting protein kinase-2, J. Exp. Clin. Cancer Res. 27 (2008) 22.
[23] W. Lee, S. Swarup, J. Chen, T. Ishitani, E.M. Verheyen, Homeodomain-interacting protein kinases (Hipks) promote Wnt/Wg signaling through stabilization of β-catenin/Arm and stimulation of target gene expression, Development 136 (2009) 241–251.
[24] S.H. Louie, X.Y. Yang, W.H. Conrad, J. Muster, S. Angers, R.T. Moon, B.N. Cheyette, Modulation of the β-catenin signaling pathway by the dishevelled-associated protein Hipk1, PLoS One 4 (2009) e4310.
[25] K. Isono, K. Nemoto, Y. Li, Y. Takada, R. Suzuki, M. Katsuki, A. Nakagawara, H. Koseki, Overlapping roles for homeodomain-interacting protein kinases hipk1 and hipk2 in the mediation of cell growth in response to morphogenetic and genotoxic signals, Mol. Cell. Biol. 26 (2006) 2758–2771.
[26] Y. Aikawa, L.A. Nguyen, K. Isono, N. Takakura, Y. Tagata, M.L. Schmitz, H. Koseki, I. Kitabayashi, Roles of HIPK1 and HIPK2 in AML1- and p300-dependent transcription, hematopoiesis and blood vessel formation, EMBO J. 25 (2006) 3955–3965.