ARTICLE
Journal of Cellular Biochemistry 116:170–178 (2015)

Journal of Cellular Biochemistry

HN1 Negatively Influences the β-Catenin/E-Cadherin Interaction, and Contributes to Migration in Prostate Cells

Lokman Varisli,[1,2]* Bilge E. Ozturk,[1] Gencer K. Akyuz,[1] and Kemal S. Korkmaz[1]

[1]*Department of Bioengineering, Faculty of Engineering, Cancer Biology Laboratory, Ege University, Bornova, Izmir, Turkey*

[2]*Department of Biology, Art and Science Faculty, Harran University, Osmanbey Campus, Sanliurfa, Turkey*

ABSTRACT

Previously, it has been reported that HN1 is involved in cytoplasmic retention and degradation of androgen receptor in an AKT dependent manner. As HN1 is a hormone inducible gene, and has been shown that it is upregulated in various cancers, we studied the importance of HN1 function in β-catenin signaling in prostate cancer cell line, PC-3 and mammary cancer cell line MDA-MB231. Here, we demonstrated that HN1 physically associates with GSK3β/β-catenin destruction complex and abundantly localizes to cytoplasm, especially when the GSK3β is phosphorylated on S9 residue. Further, ectopic HN1 expression results an increase in the β-catenin degradation leading to loss of E-cadherin interaction, concurrently contributing to actin re-organization, colony formation and migration in cancer cell lines. Thus, we report that HN1 is an essential factor for β-catenin turnover and signaling, augments cell growth and migration in prostate cancer cells. J. Cell. Biochem. 116: 170–178, 2015.

KEY WORDS: HN1; GSK3β; β-CATENIN; ADHERENT JUNCTION

β-Catenin is a multifunctional protein that is involved in many cellular processes in oncogenic signaling. Its accumulation is associated with cellular growth with its well-characterized transcriptional function [Novak and Dedhar, 1999; Moon et al., 2002]. In cellular growth processes, two important transcriptional targets of β-catenin are described as c-myc and cyclin D1 previously [He et al., 1998; Shtutman et al., 1999]. Furthermore, β-Catenin exhibits important roles in the cytoplasm interacting with Cadherin and actin cytoskeleton in plasma membrane [Ben-Ze′ev et al., 2000; Nelson and Nusse, 2004] through actin fibers and/or microtubules during cell division [Ozawa et al., 1989; Dietrich et al., 2002; Kaplan et al., 2004; Bahmanyar et al., 2008]. As, the β-Catenin protein level is tightly controlled by ubiquitination-dependent proteasomal degradation [Barth et al., 1999; Berx and Van, 2001], in the absence of inhibitory signaling, such as Wnt or PI3K/AKT, free β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β) destruction complex, which is mainly composed of GSK3β, APC, and Axin, and rapidly targeted for proteosome machinery [Woodgett, 2001, 2003]. Consistently, it was also reported that GSK3β inhibition via phosphorylation at the Serine 9 site increases β-catenin accumulation [Delcommenne et al., 1998; Sharma et al., 2002]. Further, it is believed that uncontrolled cell cycle progression is directly influenced by alterations in β-catenin, as the expression of a stabilized mutant β-catenin in a non-transformed cell line confers only a minor anchorage-independent growth advantage in soft agar assays [Barth et al., 1999]. These findings suggest that there are additional negative effects of β-catenin during cellular transformation apart from augmenting the development of aggressive cancer phenotype. Therefore, identification of novel regulators of β-catenin parallel to its transcriptional function is essential for improving our understanding of β-catenin-mediated signaling and downstream events in cancer progression. Since, cytoplasmic membrane-localized β-catenin interacts with E-cadherin and establishes a functional bridge between adherent junctions and the actin cytoskeleton, the molecules that are involved in the regulation of β-catenin level and signaling might be novel therapeutic targets to control the cancer cell migration and invasion.

The *HN1* gene encodes a small, 154-aa protein in humans, and no functional domains or motifs were identified in its primary structure. Further, the *HN1*-encoded protein has no identified homolog other than HN1L; it only shares 30% identity. Hence, HN1 is ubiquitously expressed in vertebrates and is evolutionarily conserved [Zhou et al.,

Grant sponsor: The Scientific and Technological Research Council of Turkey (TUBITAK); Grant numbers: 108S288, 110S134, 113Z083.

*Correspondence to: Lokman Varisli, PhD, Department of Biology, Art and Science Faculty, Harran University, Sanliurfa, Turkey. E-mail: lokmanv@gmail.com

Manuscript Received: 2 March 2014; Manuscript Accepted: 22 August 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 28 August 2014

DOI 10.1002/jcb.24956 • © 2014 Wiley Periodicals, Inc.

2004; Varisli et al., 2011], siRNA-mediated depletion of HN1 in both melanoma and prostate cells results in delayed G1/S-phase transition and increases the p21, cyclin D1, and cyclin B1 levels, suggesting that HN1 significantly contributes to the cell cycle regulation [Laughlin et al., 2009; Varisli et al., 2011]. Moreover, gene expression analysis and in vivo studies demonstrated that *HN1* mRNA also increases during wound healing and retina regeneration [Zujovic et al., 2005; Goto et al., 2006], and the cells ectopically overexpressing HN1 heal wounds faster than controls [Varisli et al., 2012]. In our previous studies, we showed that HN1 involves in the regulation of GSK3β phosphorylations via downregulating the PI3K/AKT signaling. Therefore, the cellular level of HN1 influences β-catenin stability and the transactivation of β-catenin targets in prostate cells [Varisli et al., 2011], thus, we suggest that HN1 is an important regulator of GSK3β-dependent interactions of β-catenin and E-cadherin and, furthermore, downregulates the Epidermal Growth Factor Receptor (EGFR)-mediated signaling in prostate cancer cell line PC-3 that remains to be studied in detail.

In this study, we demonstrate that HN1 is a novel interaction partner of the inactive GSK3β/β-catenin/APC complex, but not of the active complex. HN1 contributes to ubiquitin-dependent proteasomal degradation of β-catenin, subsequently the turnover via facilitating the GSK3β phosphorylation. Accordingly, overexpression of HN1 decreases the E-cadherin interaction of β-catenin; contrary its depletion remarkably stabilizes the β-catenin level and its interaction with E-cadherin. Furthermore, we also found that increased HN1 expression augments colony formation in both control and EGFRi-treated cells. Overall, our data suggest that HN1 functions as a downstream regulator of EGFR-dependent colony formation. In addition to its regulatory role in cell cycle progression, HN1 also contributes to cell migration through controlling the stability of β-catenin interaction with E-cadherin in adherent junctions. Increased HN1 expression might result with the loss of these interactions that leads to the increased migration, eventually cancer progression.

MATERIALS AND METHODS

CELL CULTURE AND PROPAGATION

PC-3 and MDA-MB231 cells were obtained from American Type Culture Collection (ATCC Manassas), and propagated as recommended in DMEM/F12 medium supplemented with 5% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) in humidified atmosphere with 5% CO_2 at 37°C.

CONSTRUCT, siRNA, AND ANTIBODIES

The full-length open reading frame of human HN1 cDNA was used for the construction of pcDNA4-HN1 wild type (154 aa), as described previously [Varisli et al., 2011, 2012]. Antibodies for HN1 were either produced as described previously [Varisli et al., 2011] or purchased from Sigma (Europe) and GeneTex (Irvine, CA). β-Catenin, pGSK3β$^{(S9)}$, pGSK3β$^{(Y216)}$, E-cadherin, TCF-4, APC, ubiquitin, and non-specific IgG antibodies were purchased from Santa Cruz, Inc. (Bergheim, Germany). Alexafluor 488 and 594 conjugated secondary antibodies were also purchased from Invitrogen (Carlsbad, CA). Control or HN1 specific siRNAs [Varisli et al., 2011] were purchased from Santa Cruz, Inc.

TRANSFECTIONS

All plasmid (pcDNA4-GFP-HN1, pcDNA4-HM or pcDNA4-HM-HN1) and siRNA (scrambled or HN1) transfections were performed using Fugene HD transfection reagent (Promega, UK), as we described previously [Varisli et al., 2011, 2012]. During the silencing experiments, siRNA transfections significantly reduced HN1 expression (70–80% depletion) for 72 h, which agrees with previous results [Varisli et al., 2011, 2012].

IMMUNOBLOTTING AND IMMUNOPRECIPITATION (IP)

Lysates were prepared, and 0.5 mg of protein lysate was used for each immunoprecipitation experiment. Following 1 h pre-clearance of lysates using 40 μl IP matrix (Santa Cruz, Inc.), lysates were divided into two tubes and incubated for 4 h using either a ubiquitin specific antibody or non-specific IgG. Then, 40 μl of IP matrix was added to the lysate-antibody complex and incubated overnight to collect complexes. Next the samples were washed two times using RIPA, and the precipitates were denatured in 25 μl Laemmli buffer at 95°C for 5 min. Then, the immunoblotting procedure was performed as described previously [Varisli et al., 2011, 2012]. Cytosolic and non-cytosolic (nuclear + membrane) protein lysates were prepared using as previously described [Varisli et al., 2012].

F-ACTIN AND IMMUNOFLUORESCENCE (IF) LABELING

Alexafluor 488-conjugated phalloidin (Invitrogen) was used to label F-actin using immunofluorescence microscopy. IF labeling was performed as described previously [Varisli et al., 2011, 2012]. Finally, the cells were mounted onto slides with 30% glycerol in PBS supplemented with 0.5 μg/ml DAPI and analyzed immediately using a DMIL fluorescent microscope (Leica, Germany). Images were captured using 5.5 MPix digital camera, and processed with image software.

ANALYSIS OF β-CATENIN PROTEIN STABILITY

The cells were transfected with empty vector (pcDNA4-HM) or HN1-expressing plasmid (pcDNA4-HM-HN1) for 24 h. After transfections, cells were treated with MG132 (10 μM, Sigma) or vehicle (DMSO) for an additional 24 h. Then, cell lysates were prepared and subjected to immunoblot analyses using anti-HN1, -β-catenin, and -β-actin antibodies. Cytosolic proteins were separated from membrane and nuclear compartments using low salt (50 mM HEPES pH: 7.4, 10 mM KCl, 1 mM EDTA, 1 mM EGTA) and high salt (50 mM HEPES pH: 7.4, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, and % 0.5 Triton X-100) extraction method respectively. To study the protein turnover, cyclohexamide (10 μM) treatments were performed for the indicated time points.

COLONY FORMATION ASSAY

Colony formation was performed as described previously [Korkmaz et al., 2005; Varisli et al., 2012] and the area covered on each plate by the colonies was measured using colony counter software [Wouters et al., 2010]. The software thresholds were adjusted to count colonies that were $\geq 2\,mm^2$, and the graphs were constructed using the obtained values.

SCRATCH ASSAY

PC-3 and MDA-MB231 cells were transfected with empty vector (pcDNA4-HM) or HN1-expressing plasmid (pcDNA4-HM-HN1) for 24 h. After transfections, plates were scratched using 1 ml pipette tip (end was cut) and inhibitor was added. Then, the scratches were visualized at every 24 h as indicated time points. Three images were taken from each scratch and the experiments were replicated three times to draw graphs.

RESULTS

HN1 PLAYS A ROLE IN THE REGULATION OF GSK3β PHOSPHORYLATION AND β-CATENIN TURNOVER

GSK3β is a constitutively active Serin/Threonin kinase that phosphorylates β-catenin to facilitate its degradation. However, the activity of GSK3β can be suppressed by phosphorylation at Serine 9 by multiple pathways, including EGFR. This inhibitory phosphorylation of GSK3β promotes the accumulation of β-catenin upon activation of the PI3K-Akt signaling axis. In our previous studies, we have shown that the activity of AKT is downregulated in HN1 overexpressing cells [Varisli et al., 2011, 2012]. Therefore, here, we examined the role of HN1 onto GSK3β phosphorylations and function, subsequently the altered β-catenin level in prostate cancer cell line, PC-3. We observed that β-catenin level was decreased by HN1 overexpression, and this was blocked significantly by MG132 treatment (Fig. 1A). Since, ubiquitin dependent proteosomal degradation of β-catenin is mainly controlled by phosphorylation, we investigated the GSK3β inhibition using specific inhibitor SB216763 and found that inhibitor-mediated GSK3β inhibition resulted β-catenin accumulation in nuclear and cytosolic extracts while the HN1 level was remarkably decreased (Fig. 1B). When we investigated the S33 phosphorylation of β-catenin, we observed that it was increased in HN1 transfected cells in comparison to vector controls (Fig 1C). To understand whether the decrease in β-catenin

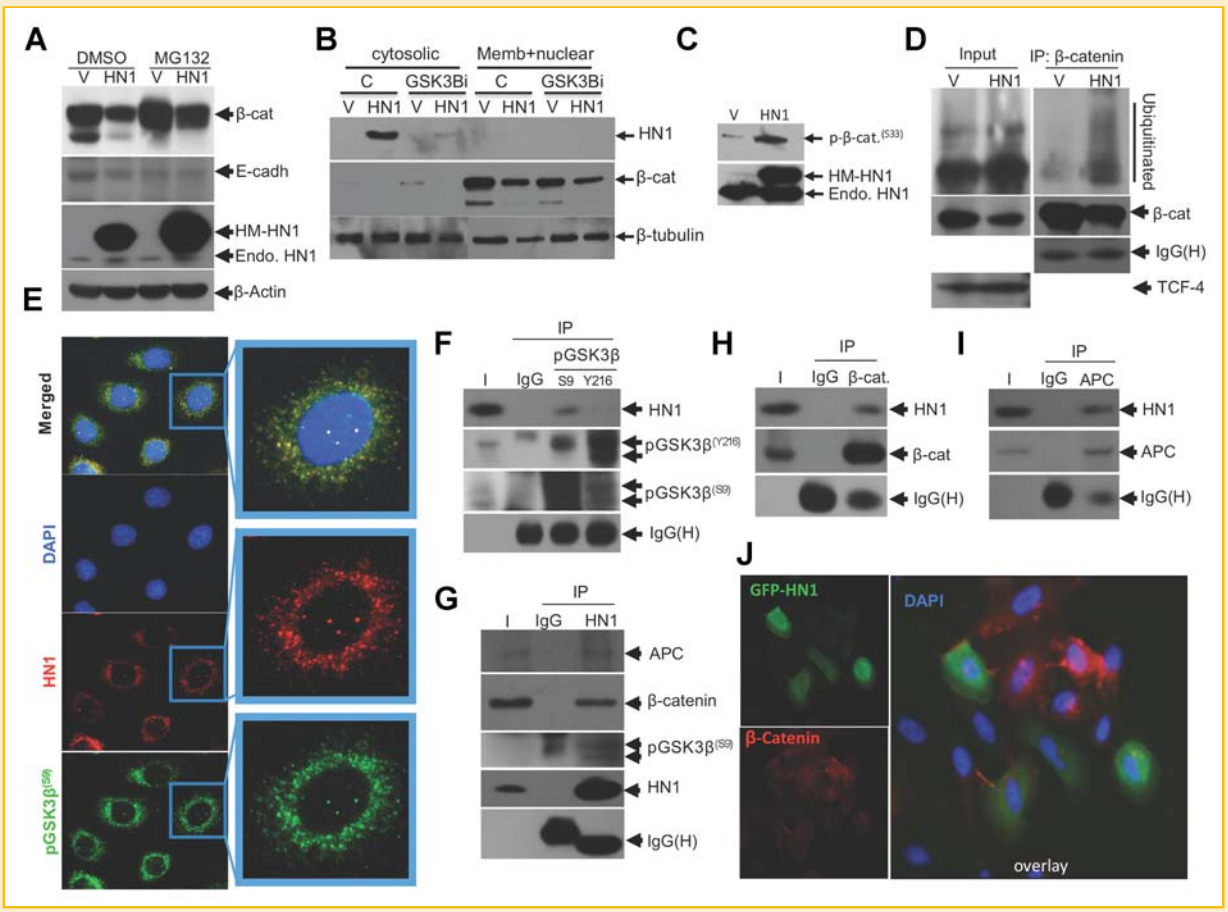

Fig. 1. HN1 regulates β-catenin level by direct association. A: Proteosome inhibitor MG132 restores the β-catenin level but not E-cadherin in HN1 overexpressed cells. Endogenous HN1 expression can also be seen in PC-3 cells. B: SB216763-mediated GSK3β inhibition resulted both loss of HN1 expression and nuclear β-catenin accumulation in nuclear and cytosolic extracts. C: β-catenin$^{(S33)}$ increased in HN1 transfected cells in comparison to vector controls. D: Ectopic HN1 expression facilitates ubiquitination of β-catenin, which is demonstrated by β-catenin-specific ubiquitin immunoprecipitation-immunoblot analyze. Further, E: HN1 (red) and pGSK3β$^{(S9)}$ (green) co-localize within cytoplasm as speckles in PC-3 cells. HN1 specific IPs evidence that the HN1 interacts with F GSK3β$^{(S9)}$ but not with $^{(Y216)}$, G, H, and I: β-catenin as well as APC reciprocal immunoprecipitations also confirm these associations. TCF4 and IgGs from precipitations were also labeled, and shown for comparison. Western blots were performed three times, and IPs twice. J: The GFP-HN1 expressed cells exhibit lower levels of β-catenin expression than untransfected PC-3 cells. β-catenin was labeled using Alexafluor 594 and the DAPI was used for nuclear staining.

expression level is predominantly related to inhibition of protein synthesis or proteasomal degradation, we treated the cells with Cycloheximide (CHX) and MG132 in vector or HN1 transfected PC-3 cells respectively (Supplementary Fig. S1). The results showed that β-catenin level was decreased during overexpression of HN1, and this was blocked by MG132 treatment significantly in concordance with previous findings (Fig. 1B and Supplementary Fig. S1). Also, the CHX treatment decreased the β-catenin level regardless of HN1; the data suggested that the HN1 influence on β-catenin level is not post-transcriptional but proteasomal degradation dependent. Further, when lysates from vector- or HN1-transfected PC-3 cells were subjected to immunoprecipitation-immunoblot analyses using β-catenin and ubiquitin antibodies, it was observed that the β-catenin ubiquitination was higher in HN1 expressing cells than vector controls (Fig. 1D). This data demonstrated that the degradation of β-catenin was ubiquitination-dependent via increased S33 phosphorylation and this is mediated by HN1 through suppressing the inhibitory S9 phosphorylation of GSK3β perhaps leading to its activation in PC-3 prostate cancer cells.

To investigate the putative co-localizations of HN1 and pGSK3β$^{(S9)}$, their sub-cellular localizations were examined in PC-3 cells by immunofluorescence microscopy. We found that HN1 co-localized with pGSK3β$^{(S9)}$ (Fig. 1E). To assess whether this could be due to a physical association of both proteins, GSK3β was also immunoprecipitated using phospho-specific antibodies for pGSKβ$^{(S9)}$ and $^{(Y216)}$ at native conditions, and followed by immunoblot analysis for HN1. The results demonstrated that HN1 predominantly interacted with inactive pGSK3β$^{(S9)}$ rather than the active form of pGSK3β$^{(Y216)}$ (Fig. 1F), suggesting that HN1 maintains the activated state of the GSK3β, which is a major kinase of the cytoplasmic APC/β-catenin/Axin complex. Hence, HN1 also associates physically with β-catenin, we also investigated the HN1 interactions with other components of the complex such as APC (Fig. 1G). Furthermore, the reciprocal IPs were also performed using anti-HN1 antibody, and all interactions were validated once more (Fig. 1H,I). Furthermore, we observed that the cells exhibited lower levels of β-catenin expression/distribution when the PC-3 cells were transfected with GFP-HN1 than untransfected PC-3 cells (Fig. 1J). Thus, the co-localization and immunoprecipitation data support that HN1 physically associates with GSK3β/β-catenin/APC, and the HN1 level is an important balancing factor for stabilization of the complex. Increased HN1 expression advances the degradation of β-catenin, which consequently represses the function of the GSK3β/β-catenin/APC complex.

HN1 CONTROLS β-CATENIN/E-CADHERIN INTERACTION AT THE PROTEIN LEVEL

It is well known that sub-cellular β-catenin also localizes to membrane, and is a major component of adherent junctions interacting to E-cadherin [Nelson and Nusse, 2004]. Therefore, we examined the effect of HN1 expression onto β-catenin and E-cadherin interaction, and found that this association was significantly reduced when the HN1 was overexpressed in cells comparison to controls (Fig. 2A). However, subsequent immunoblot analysis for TCF4, the sole nuclear partner of β-catenin did not result significant change in its interaction (Fig. 2A). In concordance, we found that there is a considerable increase in β-catenin and E-cadherin interaction in HN1 depleted cells comparison to controls (Fig. 2B). Overall, our data suggest that HN1 facilitates the ubiquitin-mediated degradation of cytoplasmic β-catenin, which shuttles between adherent junctions and the β-catenin destruction complex.

As, the depletion or overexpression of HN1 remarkably alters the physical association of E-cadherin and β-catenin and the β-catenin is a key molecule for the attachment of the actin cytoskeleton to the plasma membrane at adherent junctions, we further examined the role of HN1 in organization of the actin cytoskeleton. We labeled the F-actin in control (scrambled siRNA) or HN1 depleted cells with Alexafluor 488-conjugated phalloidin, and observed that the HN1-

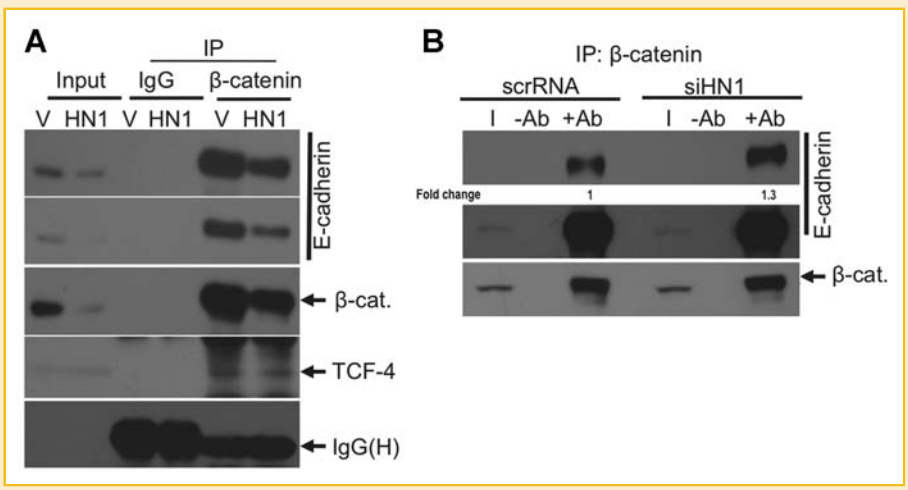

Fig. 2. HN1 influences β-catenin interaction with E-cadherin negatively. A: Ectopic HN1 expression suppresses the β-catenin interaction with E-cadherin in PC-3 cells, whereas it does not appear to influence the interaction between β-catenin and TCF-4. B: The HN1 depletion using siRNA increases the β-catenin–E-cadherin interaction in contrast to ectopic expression data. IPs and blots were performed twice in each experiment following ectopic expressions or knockdowns.

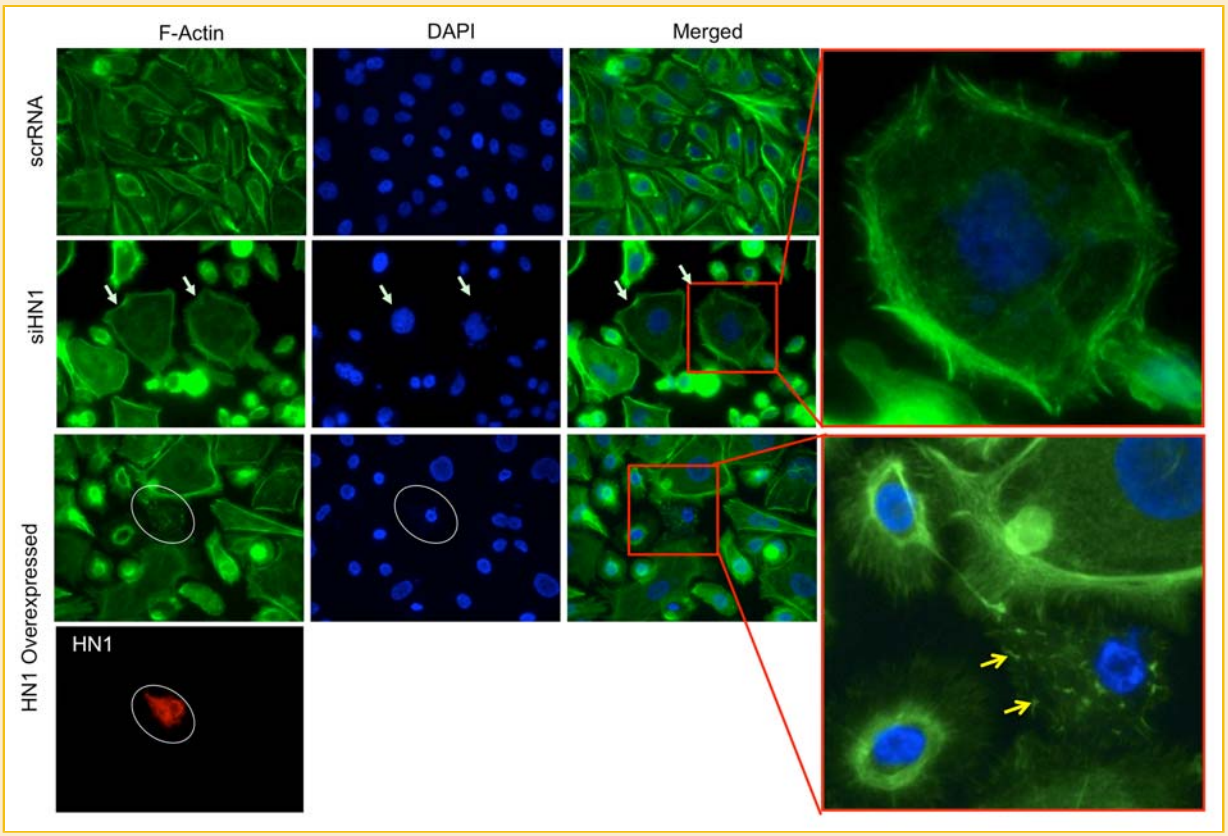

Fig. 3. HN1 is involved in organization of the actin cytoskeleton. Intriguingly, HN1 depletion results protein accumulations, evidenced as enlargement in cells (arrows indicate the cells with larger cytoplasm, and also with nuclei). In contrast, ectopic expression of HN1 disrupts the accumulation of F-actin fibers, and augments the formation of short, perhaps broken F-actin fibers in cell's cytoplasm (shown with yellow arrows in larger image).

depleted cells exhibited more amorphous and multi-foci nuclei in comparison to controls, as reported previously (arrows, Fig. 3) [Varisli et al., 2011]. Moreover, F-actin prominently accumulated at the plasma membrane in these cells comparison to controls. In contrast, HN1 overexpression resulted in disrupted F-actin distribution that was characterized by shorter stress fibers (Fig. 3) characterized by a reduction in β-catenin expression level due to its increased degradation as aforementioned above (Fig. 1). Thus, the β-catenin level is important for cytoskeletal organization of actin, and is facilitated by HN1 level in PC-3 cells.

HN1 CONTRIBUTES TO TUMOR GROWTH AND SYNERGIZES WITH EGFR SIGNALING

The loss of E-cadherin expression is a common event in several cancers, and decreases the interaction with β-catenin that promotes more aggressive cancer phenotypes. We investigated whether HN1 expression might affect in vitro tumor formation in the presence of EGFRi or the absence of EGF. Consistent with our previous studies [Varisli et al., 2012], we found that the overexpression of HN1 resulted with a significant increase in colony formation in PC-3 cells ($P < 0.05$) that was suppressed by EGFRi treatment. However, HN1 overexpression partially restored the capacity for colony formation (Figs. 4A and B), whereas its depletion decreased the number of colonies formed by both control and EGF-treated cells (Figs. 4C and D). Interestingly, in our further studies with GSK3β inhibitor SB216763, we found that HN1-mediated migration of PC-3 cells were suppressed when using 5 μM inhibitor (Figs. 5A and B) whereas the migration with HN1 expression was not effected in MDA-MB231 cells (Figs. 5C and D). The results were obtained from scratch assay for validating the cellular migration. Since the EGFR-mediated AKT activation leads to GSK3β inhibition via S9 phosphorylation, and the EGFRi treatment decreases colony formations that is augmented by HN1, therefore, we suggest that the GSK3βi-mediated inhibition of migration might be consequent to decreased HN1 expression in PC-3 cells (Fig. 1B) but not in EGFR negative MDA-MB231 cells. Taken together, the data indicate that the increased expression of HN1 synergizes with EGF signaling to augment colony formation in PC-3 cells. Therefore, HN1 might play an important role in the EGF-dependent carcinogenesis in prostate cells.

DISCUSSION

HN1 is a novel protein with putative cellular functions, which are not fully understood yet. The knockdown of HN1 level impairs cell cycle progression in prostate and melanoma cells [Laughlin et al., 2009;

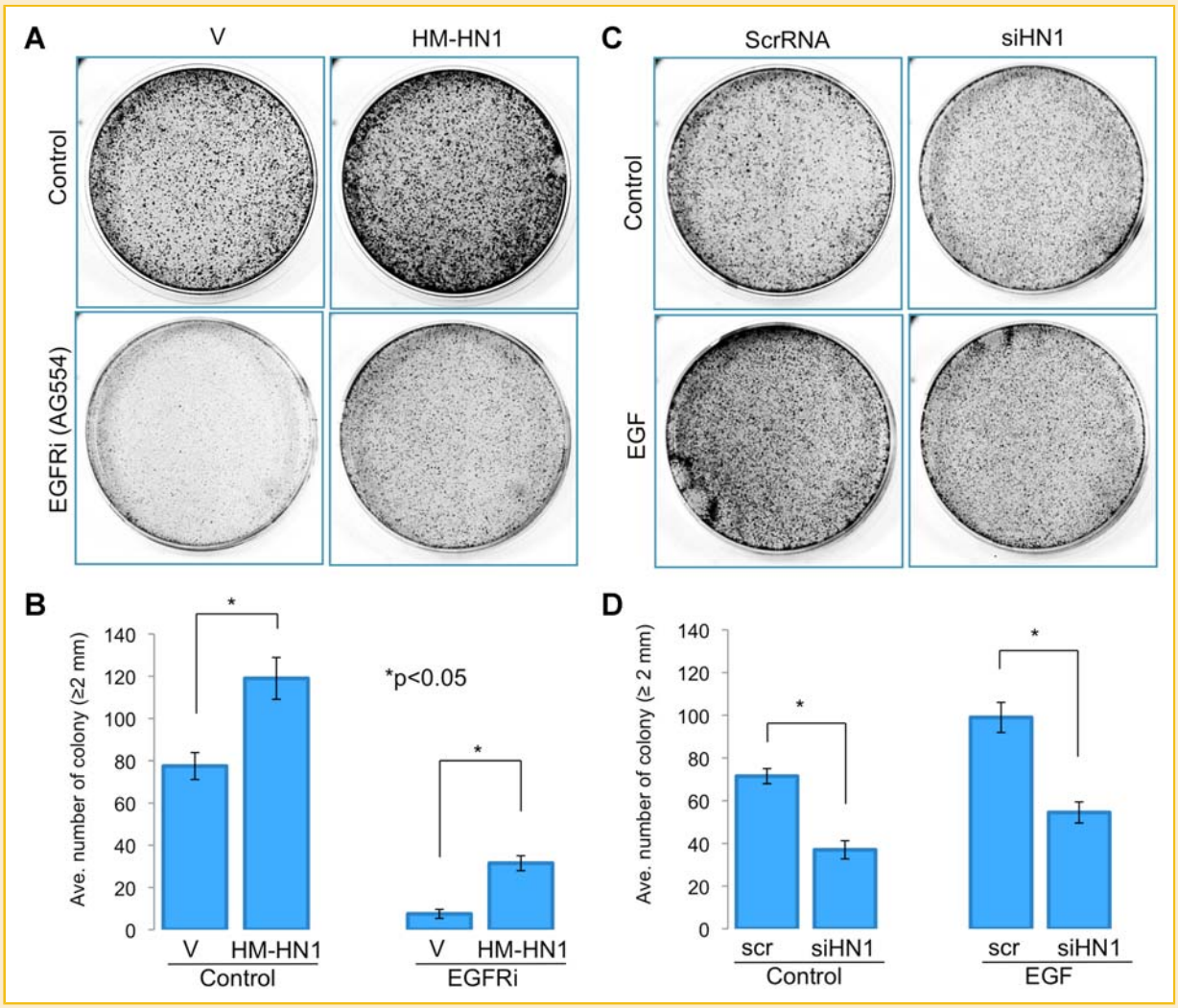

Fig. 4. HN1 contributes to colony formation in PC-3 cells. A: Ectopic HN1 expression increases the number of colonies in both control and EGFR inhibitor-treated cells compared to vector-transfected controls. Average colony numbers are illustrated as histogram plots for comparison ($P < 0.05$). C–D: Additionally, HN1 depletion reduces the number of colonies formed despite the EGF treatment. Average colony numbers are also provided as histogram plots for comparison ($P < 0.05$).

Varisli et al., 2011], however, HN1 mRNA level was reported to be high in various carcinomas, while low in benign tumors of yolk sac, gliomas, and teratomas [Varisli et al., 2011]. Whether there is a link between the malignant potential of carcinomas and HN1 expression, HN1-regulated turnover of β-catenin and the E-cadherin–β-catenin interaction affecting colony formation and cellular growth in PC-3 prostate as well as MDA-MB231 mammary cell lines were examined. Here, we report that HN1 is involved in the regulation of β-catenin turnover via facilitating an increase in suppression of GSK3β phosphorylations leading its activation, thus influencing the β-catenin interaction with E-cadherin in adherent junctions. Consistently, a significant nuclear and cytosolic accumulation of β-catenin was also observed consequent to HN1 depletion concurrent to GSK3β inhibition when a specific inhibitor SB216763 was used. The data suggest that there could a feedback regulation in between GSK3β and HN1 level, in which suppression of kinase function by inhibitor leads to a significant decrease in HN1 level. This requires further studies.

β-Catenin level is primarily regulated via ubiquitin-dependent proteasomal degradation upon phosphorylations on amino acid residues S33, S37, S41, and S45 by CK1 and GSK3β [Aberle et al., 1997; Orford et al., 1997]. In the absence of an inhibitory signal, such as Wnt and growth factors, β-Catenin turnover is regulated through ubiquitination by β-Trcp. As the β-Catenin localizes into membrane abundantly, also it is observed in cytoplasmic complex together with scaffolding proteins, such as Axin, APC, CK1, and GSK3β [Rubinfeld et al., 1993; Siegfried et al., 1994; Ikeda et al., 1998; Kishida et al., 1998], while the destruction complex is not disrupted yet [Aberle et al., 1997; Amit et al., 2002]. However, altered expression of β-catenin, which is usually stabilized in cytoplasm has been reported in several human cancers, including gastric, colon, hepatocellular, and esophageal tumors and prostatic

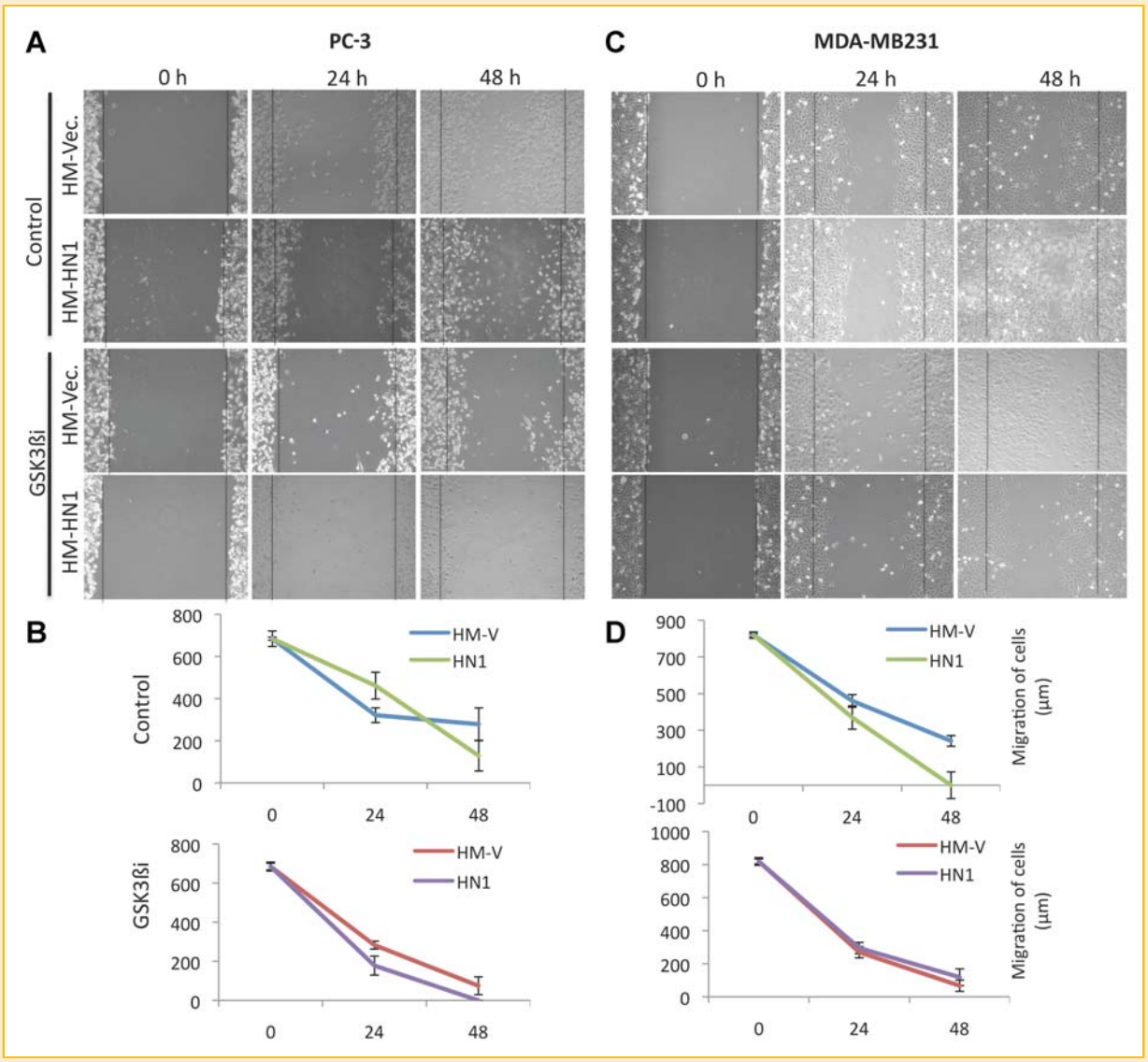

Fig. 5. HN1 augments migration in PC-3 and MDA-MB231 cells. Ectopic HN1 expression increases the rate of migration in control but not in GSK3β inhibitor-treated cells compared to vector-transfected A: PC-3 and C: MDA-MB231. B: and D: Average migration rates are illustrated as linear plots for comparison ($P < 0.05$).

intraepithelial neoplasias (PINs) [Morin, 1999; Polakis, 1999; Gounari et al., 2002]. Hence, its localization in the adherent junctions contradicts the finding that β-catenin is an important factor for increasing the proliferation rate of cancer cells, the ectopic expression studies suggest that β-catenin also restores the impaired cell–cell contacts, which was examined in human gastric cancer cell lines [Oyama et al., 1994]. In addition, the disrupted interaction between E-cadherin and β-catenin in adherent junctions promotes more invasive and aggressive cancer phenotypes [Hajra and Fearon, 2002] was consistent with our data and previous reports. Therefore, we suggest that increased HN1 expression suppresses inactive GSK3β level through leading to a decrease in S9 phosphorylation that is mediated by AKT pathway EGF-dependently [Varisli et al., 2011]. This also needs interaction studies to be examined in detail.

E-cadherin is a large calcium-dependent glycoprotein that contains an extracellular single-pass trans-membrane domain with a short conserved cytoplasmic tail [Berx and Van, 2001]. It exerts a potent invasion-suppressing effect in both tumor cell lines and in vivo tumor models [Frixen et al., 1991; Perl et al., 1998]. The ectopic expression of E-cadherin decreases the proliferation of several mammary carcinoma cell lines [St Croix et al., 1998]. Poorly differentiated tumors exhibit reduced E-cadherin expression; consistently well-differentiated tumors exhibit higher expression [Frixen et al., 1991; Figarella-Branger et al., 1994]. In addition to β-catenin, HN1 also contributes to E-cadherin stability and down-regulates its level when overexpressed (Fig. 2B). However, we could not found any physical interaction between HN1 and E-cadherin (data not shown). It is possible that HN1 may influence interactions between E-cadherin and other catenin proteins. Consequently, HN1

level changes interactions between cadherin and catenin proteins and this may be important for development, cell polarity, integrity and tissue morphology, as known functions of cadherin and catenin proteins [Takeichi, 1991]. Since, disruption of the cadherin–catenin complex has been found in carcinomas of different origins and correlated with various pathologic and clinical features, such as tumor differentiation, infiltrative growth, lymph node metastasis, and poor prognosis [Hajra and Fearon, 2002], expression of functional E-cadherin in cells suppresses the invasive behavior without endogenous E-cadherin [Vleminckx et al., 1991]. Thus, the data suggest that HN1 expression induces a decrease in both E-cadherin and β-catenin levels, consequently the interactions, whereas the HN1 depletion restores the levels and localizations of both proteins, as well as the interactions (Fig. 2B). Furthermore, since the β-catenin is an important component of the cytoskeleton and localizes to the adherent junctions through E-cadherin interaction, HN1-mediated expression alteration in stress fiber formation was also examined in our studies. Then, we have evidenced that the F-actin abundantly localizes to the membrane that is disrupted when the HN1 is overexpressed in these cells. Likewise, HN1 depletion results with altered nuclear morphology concurrent to elevated β-catenin level [Varisli et al., 2011]. Thus, the loss of membrane-localized β-catenin presumably augments the EGFR-mediated carcinogenesis [Frixen et al., 1991; Perl et al., 1998; Varisli et al., 2011] and contributes to disruption of cell cycle control that needs to be verified in further studies.

Taken together, HN1 expression exerts a marked influence on β-catenin level and F-actin organization that might be mediated through the disrupted interaction between E-cadherin and β-catenin. Because, the HN1 destabilizes the GSK3β/β-catenin/APC destruction complex and associates with pGSK3β$^{(S9)}$ but not with pGSK3β$^{(Y216)}$, our data collectively indicate that HN1 is a novel interaction partner of inactive β-catenin complex and contributes to cytoskeleton dynamics therefore the migration ability of the cells. Whether the effects are perhaps mediated through the translocation of β-catenin from the plasma membrane to the cytoplasm needs to be clarified in further studies.

ACKNOWLEDGMENTS

This research was supported by project grants from TUBITAK 108S288 (COST_CANGENIN), 110S134, and 113Z083 to K.S.K.

REFERENCES

Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. 1997. Beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J 16:3797–3804.

Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, Mann M, Ben-Neriah Y, Alkalay I. 2002. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: A molecular switch for the Wnt pathway. Genes Dev 16:1066–1076.

Bahmanyar S, Kaplan DD, Deluca JG, Giddings TH, Jr., O'Toole ET, Winey M, Salmon ED, Casey PJ, Nelson WJ, Barth AI. 2008. Beta-Catenin is a Nek2 substrate involved in centrosome separation. Genes Dev 22:91–105.

Barth AI, Stewart DB, Nelson WJ. 1999. T cell factor-activated transcription is not sufficient to induce anchorage-independent growth of epithelial cells expressing mutant beta-catenin. Proc Natl Acad Sci U S A 96:4947–4952.

Ben-Ze′ev A, Shtutman M, Zhurinsky J. 2000. The integration of cell adhesion with gene expression: The role of beta-catenin. Exp Cell Res 261:75–82.

Berx G, Van Roy F. 2001. The E-cadherin/catenin complex: An important gatekeeper in breast cancer tumorigenesis and malignant progression. Breast Cancer Res 3:289–293.

Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S. 1998. Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. Proc Natl Acad Sci U S A 95:11211–11216.

Dietrich C, Scherwat J, Faust D, Oesch F. 2002. Subcellular localization of beta-catenin is regulated by cell density. Biochem Biophys Res Commun 292:195–199.

Figarella-Branger D, Pellissier JF, Bouillot P, Bianco N, Mayan M, Grisoli F, Rougon G. 1994. Expression of neural cell-adhesion molecule isoforms and epithelial cadherin adhesion molecules in 47 human meningiomas: Correlation with clinical and morphological data. Mod Pathol 7:752–761.

Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W. 1991. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 113:173–185.

Goto T, Hisatomi O, Kotoura M, Tokunaga F. 2006. Induced expression of hematopoietic- and neurologic-expressed sequence 1 in retinal pigment epithelial cells during newt retina regeneration. Exp Eye Res 83:972–980.

Gounari F, Signoretti S, Bronson R, Klein L, Sellers WR, Kum J, Siermann A, Taketo MM, von Boehmer H, Khazaie K. 2002. Stabilization of beta-catenin induces lesions reminiscent of prostatic intraepithelial neoplasia, but terminal squamous transdifferentiation of other secretory epithelia. Oncogene 21:4099–4107.

Hajra KM, Fearon ER. 2002. Cadherin and catenin alterations in human cancer. Genes Chromosomes Cancer 34:255–268.

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. 1998. Identification of c-MYC as a target of the APC pathway. Science 281:1509–1512.

Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. 1998. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J 17:1371–1384.

Kaplan DD, Meigs TE, Kelly P, Casey PJ. 2004. Identification of a role for beta-catenin in the establishment of a bipolar mitotic spindle. J Biol Chem 279:10829–10832.

Kishida S, Yamamoto H, Ikeda S, Kishida M, Sakamoto I, Koyama S, Kikuchi A. 1998. Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. J Biol Chem 273:10823–10826.

Korkmaz CG, Korkmaz KS, Kurys P, Elbi C, Wang L, Klokk TI, Hammarstrom C, Troen G, Svindland A, Hager GL, Saatcioglu F. 2005. Molecular cloning and characterization of STAMP2, an androgen-regulated six transmembrane protein that is overexpressed in prostate cancer. Oncogene 24:4934–4945.

Laughlin KM, Luo D, Liu C, Shaw G, Warrington KH, Jr., Law BK, Harrison JK. 2009. Hematopoietic- and neurologic-expressed sequence 1 (Hn1) depletion in B16.F10 melanoma cells promotes a differentiated phenotype that includes increased melanogenesis and cell cycle arrest. Differentiation 78:35–44.

Moon RT, Bowerman B, Boutros M, Perrimon N. 2002. The promise and perils of Wnt signaling through beta-catenin. Science 296:1644–1646.

Morin PJ. 1999. Beta-catenin signaling and cancer. Bioessays 21:1021–1030.

Nelson WJ, Nusse R. 2004. Convergence of Wnt, beta-catenin, and cadherin pathways. Science 303:1483–1487.

Novak A, Dedhar S. 1999. Signaling through beta-catenin and Lef/Tcf. Cell Mol Life Sci 56:523–537.

Orford K, Crockett C, Jensen JP, Weissman AM, Byers SW. 1997. Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. J Biol Chem 272:24735–24738.

Oyama T, Kanai Y, Ochiai A, Akimoto S, Oda T, Yanagihara K, Nagafuchi A, Tsukita S, Shibamoto S, Ito F, Takeichi M, Matsuda H, Hirohashi S. 1994. A truncated beta-catenin disrupts the interaction between E-cadherin and alpha-catenin: A cause of loss of intercellular adhesiveness in human cancer cell lines. Cancer Res 54:6282–6287.

Ozawa M, Baribault H, Kemler R. 1989. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J 8:1711–1717.

Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. 1998. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 392:190–193.

Polakis P. 1999. The oncogenic activation of beta-catenin. Curr Opin Genet Dev 9:15–21.

Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P. 1993. Association of the APC gene product with beta-catenin. Science 262:1731–1734.

Sharma M, Chuang WW, Sun Z. 2002. Phosphatidylinositol 3-kinase/Akt stimulates androgen pathway through GSK3beta inhibition and nuclear beta-catenin accumulation. J Biol Chem 277:30935–30941.

Shtutman M, Zhurinsky J, Simcha I, Albanese C, D′Amico M, Pestell R, Ben-Ze′ev A. 1999. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci U S A 96:5522–5527.

Siegfried E, Wilder EL, Perrimon N. 1994. Components of wingless signalling in Drosophila. Nature 367:76–80.

St Croix B, Sheehan C, Rak JW, Florenes VA, Slingerland JM, Kerbel RS. 1998. E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27(KIP1). J Cell Biol 142:557–571.

Takeichi M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455.

Varisli L, Gonen-Korkmaz C, Debelec-Butuner B, Erbaykent-Tepedelen B, Muhammed HS, Bogurcu N, Saatcioglu F, Korkmaz KS. 2011. Ubiquitously expressed hematological and neurological expressed 1 downregulates Akt-mediated GSK3beta signaling, and its knockdown results in deregulated G2/M transition in prostate cells. DNA Cell Biol 30:419–429.

Varisli L, Gonen-Korkmaz C, Syed HM, Bogurcu N, Debelec-Butuner B, Erbaykent-Tepedelen B, Korkmaz KS. 2012. Androgen regulated HN1 leads proteosomal degradation of androgen receptor (AR) and negatively influences AR mediated transactivation in prostate cells. Mol Cell Endocrinol 350:107–117.

Vleminckx K, Vakaet L, Jr., Mareel M, Fiers W, van Roy F. 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell 66:107–119.

Woodgett JR. 2001. Judging a protein by more than its name: GSK-3. Sci STKE re12. DOI: 10.1126/stke.2001.100.re12.

Woodgett JR. 2003. Physiological roles of glycogen synthase kinase-3: Potential as a therapeutic target for diabetes and other disorders. Curr Drug Targets Immune Endocr Metabol Disord 3:281–290.

Wouters A, Pauwels B, Lambrechts HA, Pattyn GG, Ides J, Baay M, Meijnders P, Lardon F, Vermorken JB. 2010. Counting clonogenic assays from normoxic and anoxic irradiation experiments manually or by using densitometric software. Phys Med Biol 55:N167–N178.

Zhou G, Wang J, Zhang Y, Zhong C, Ni J, Wang L, Guo J, Zhang K, Yu L, Zhao S. 2004. Cloning, expression and subcellular localization of HN1 and HN1L genes, as well as characterization of their orthologs, defining an evolutionarily conserved gene family. Gene 331:115–123.

Zujovic V, Luo D, Baker HV, Lopez MC, Miller KR, Streit WJ, Harrison JK. 2005. The facial motor nucleus transcriptional program in response to peripheral nerve injury identifies Hn1 as a regeneration-associated gene. J Neurosci Res 82:581–591.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.