Identification of the LIM Protein FHL2 as a Coactivator of β-Catenin*

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β-Catenin is a key mediator of the Wnt pathway, which plays a critical role in embryogenesis and oncogenesis. As a transcriptional activator, β-catenin binds the transcription factors, T-cell factor and lymphoid enhancer factor, and regulates gene expression in response to Wnt signaling. Abnormal activation of β-catenin has been linked to various types of cancer. In a yeast two-hybrid screen, we identified the four and a half of LIM-only protein 2 (FHL2) as a novel β-catenin-interacting protein. Here we show specific interaction of FHL2 with β-catenin, which requires the intact structure of FHL2 and armadillo repeats 1–9 of β-catenin. FHL2 co-operated with β-catenin to activate T-cell factor/lymphoid enhancer factor-dependent transcription from a synthetic reporter and the cyclin D1 and interleukin-8 promoters in kidney and colon cell lines. In contrast, coexpression of β-catenin and FHL2 had no synergistic effect on androgen receptor-mediated transcription, whereas each of these two coactivators independently stimulated AR transcriptional activity. Thus, the ability of FHL2 to stimulate the trans-activating function of β-catenin might be dependent on the promoter context. The detection of increased FHL2 expression in hepatoblastoma, a liver tumor harboring frequent β-catenin mutations, suggests that FHL2 might enforce β-catenin transactivation activity in cancer cells. These findings reveal a new function of the LIM coactivator FHL2 in transcriptional activation of Wnt-responsive genes.

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β-Catenin is a binding partner of E-cadherin in cell-cell adherens junctions and a key effector in the Wnt signaling pathway, which plays a critical role in development and homeostasis (1, 2). β-Catenin is composed of three domains: a regulatory N-terminal region followed by 12 armadillo (arm) repeats and a C-terminal transactivation domain. The N-terminal region contains serine and threonine residues whose phosphorylation signals ubiquitin-dependent degradation of cytosolic β-catenin. Phosphorylation of β-catenin is controlled by a multiprotein complex composed of tumor suppressor adenomatous polyposis coli, Axin, glycogen synthase kinase-3, and casein kinase 1α. The arm repeats in the core region mediate β-catenin interactions with a majority of partners such as E-cadherin and the transcriptional factors, T-cell factor (TCF) and lymphoid enhancer factor (LEF). Cytosolic accumulation of β-catenin leads to trans-location of the protein into the nucleus where it forms a complex with DNA-binding factors of the TCF/LEF family (3). This bipartite transcription factor complex recruits multiple transcriptional coactivators and activates TCF/LEF-dependent transcription through the C-terminal trans-activation domain of β-catenin (4).

The tight regulation of gene expression by Wnt signaling guarantees a stringent spatiotemporal coordination of downstream gene expression in response to developmental and physiological cues. It has been shown that deregulation of β-catenin, which leads to its nuclear accumulation and activation of gene expression, is implicated in the development of cancer (reviewed in Ref. 5). Among several candidate downstream target genes, β-catenin activates transcription from the promoters of c-myc, cyclin D1, the matrix metalloproteinase-7, neuronal cell adhesion molecule, and interleukin-8 (IL-8), which are frequently overexpressed in human colon carcinoma (6–11). Different mechanisms by which β-catenin promotes target gene activation have been proposed. It has been shown that β-catenin can interact directly with the TATA-binding protein in vitro (12) and with the transcription coactivator CBP/p300, which is able to bind to TATA-binding protein and transcription factor IIB, thus linking β-catenin to the RNA polymerase II machinery (13–15). Moreover, β-catenin-interacting proteins, such as the chromatin-remodeling factor Brg-1 and CBP/p300, can be involved in altering chromatin structure to allow access of RNA polymerase II (16). Here, we report the identification of four and a half of LIM-only protein 2 (FHL2) as a novel β-catenin-binding protein that possesses intrinsic trans-activation activity.

Initially cloned by its abundant expression in human heart and its down-regulated expression in rhabdomyosarcoma cells (17–19), FHL2 (also known as down-regulated in rhabdomyosarcoma LIM protein and skeletal muscle LIM protein 3) belongs to the family of LIM proteins. The LIM domain is a specialized double zinc finger protein motif, and LIM proteins play multiple roles as adapters and functional modifiers in protein interactions (20). Containing exclusively four and a half of LIM domains, FHL proteins display a high degree of homology between family members and tissue-specific expression (21, 22).

The activity of FHL2 in transcriptional regulation has been...
evidenced in recent reports demonstrating that FHL2 is a coactivator of the androgen receptor (AR) and the cAMP response element-binding protein (CREB) (21, 23). FHL2 activates gene expression through interaction with DNA-binding transcriptional factors (21, 23). A link between FHL2 and β-catenin was first suggested by the findings that both FHL2 and β-catenin interact with AR and are capable of enhancing AR-mediated androgen-dependent transcription (23–26). In this study, we show that FHL2 binds β-catenin in vitro and in vivo. Although FHL2 alone had no effect on TCF/LEF-dependent transcription, it potentiated the trans-activating activity of β-catenin on the transcription of the Wnt-responsive cyclin D1 and IL-8 promoters. FHL2 and β-catenin independently enhanced AR activity in a hormone-dependent manner, but in this context, the combined action of both proteins had only additive effects. Evidence of up-regulated FHL2 expression in primary tumors suggests that FHL2-activating function on β-catenin may be implicated in oncogenesis by further enhancing the expression of Wnt target genes.

EXPERIMENTAL PROCEDURES

Plasmids—cDNA sequences encoding β-catenin arm repeats 1–10 (amino acids 132–556) used as the bait in a yeast two-hybrid screen as well as fragments encompassing different arm repeats were inserted into pGal4 DNA-binding domain (DBD) into the p52Sad vector (a gift of Dr. P. Legrain). FHL2 full-length cDNA was isolated from a HeLa cDNA library after a two-hybrid screen with the β-catenin bait. FHL2 deletion mutants were constructed by inserting PCR-amplified fragments in-frame with the Gal4 activation domain into pACT2 (Clontech). ACT full-length cDNA cloned into pACT2 was provided by Dr. M. Morgan. pGEX-β-catenin, pGEX-FHL2, and pGEX-FHL2-N-term were constructed by inserting full-length β-catenin and FHL2 cDNAs and FHL2 sequences coding for amino acids 1–126 in frame with glutathione S-transferase (GST) into pGEX-5X-1 (Amersham Biosciences). pGHis–β-catenin and pGHis-arm1–10 (amino acids 132–100) were constructed by inserting the RGSH6 sequence at the N-terminal end of β-catenin into pSG5 (Stratagene). Expression vectors for full-length FHL2 (pcDNAFHL2), β-catenin (pcDNAβ-cat), and β-catenin T41A (pcDNAβ-catT41A) were used in transient transfection assays. The IL-8 promoter–luciferase construct used in this study contains 195 bp upstream of the transcription start site (193-IL-8-Luc) as described previously (11). The promoter with mutated TCF site at position −186 to −177 (193mt-IL-8-Luc) was used as control (11). pTOPFLASH, pPOFPFLASH, and pANTCF4 were provided by Dr. H. Clevers; pALuc (cyclin D1 promoter) was provided by Dr. R. Pestell; pMMTV-Luc was provided by Dr. P. Chambon; and pSG5-hAR (human androgen receptor) was provided by Dr. R. Pestell. Standard recombinant DNA techniques including PCR followed by sequencing were used to construct all of the plasmids.

Yeast Two-hybrid Analysis—The two-hybrid screen was performed using the mating protocol described by Frumont-Racine et al. (27). CG1945 cells transformed with pGal4 DBD-β-catenin-arm1–10 were mixed with Y187 cells transformed with a HeLa MATCHMAKER cDNA library (HL-404AH, Clontech). Transformants were selected by their ability to grow on minimal medium lacking tryptophan, leucine, and histidine. Positive clones were then confirmed in β-galactosidase overlay assay. Prey plasmids with high β-galactosidase activity were rescued in Escherichia coli, and their sequences were subsequently analyzed. Interaction domains in β-catenin and FHL2, as well as the fusion proteins constructs were cotransformed into the diploid strain CG1945/Y187 and quantitative β-galactosidase assay was performed as described previously (28).

In Vitro Binding Assay—35S-Labeled proteins were produced in vitro using TnT-coupled reticulocyte lysate system (Promega). The GST–β-catenin and GST–FHL2 fusion proteins purified from E. coli according to the manufacturer’s instructions (Amersham Biosciences) and protease inhibitor mixture (Roche Molecular Biochemicals). Beads were washed with the binding buffer, and bound proteins were eluted at Laemmli buffer at 95 °C and subjected to SDS-PAGE.

Cell Culture, Transfection, and Luciferase Assay—293, SW480, CV1, and HeLa cells were maintained in Dulbecco’s modified Eagle medium with 10% fetal bovine serum. Transient transfection of 293 cells was carried out by calcium phosphate precipitation with 50 ng of β-catenin T41A, 0.25–1 μg of FHL2, 250 ng of pANTC4, and 0.5 μg of pTOPFLASH or pPOFPFLASH or with 250 ng of β-catenin T41A, 0.25–1 μg of FHL2, and 0.5 μg of pCyclinD1-Luc or 0.25 μg of pIL-8-Luc. SW480 cell transfection was performed using calcium phosphate precipitation with 0.25 or 1 μg of FHL2, 250 ng of pANTC4, and 0.5 μg of pTOPFLASH or pPOFPFLASH. CV1 cells were cotransfected with 0.5 μg of pMMTV-Luc, 50 ng of pSG5-hAR, 0.5 μg of β-catenin, and 0.25 μg of FHL2 using LipofectAMINE PLUS (Invitrogen). For AR transcriptional activity assays, CV1 cells were washed 3 h after transfection and cultured in Dulbecco’s modified Eagle medium supplemented with 10% stripped fetal calf serum in the presence or absence of 10 nM dihydrotestosterone (DHT)/(Sigma). The total amount of transfected DNA was kept constant by adding pCDNA3. Each transfection was performed in duplicate and repeated at least three times. A thymidine kinase–β-galactosidase plasmid was cotransfected to normalize luciferase activity for transfection efficiency. However, because FHL2 was found to activate transcription of this reporter, it could not be used for normalization; the results were confirmed by multiple independent assays.

Antibodies, Coimmunoprecipitation, and Immunofluorescence—A polyclonal anti-FHL2 antibody was generated by injection of GST–FHL2N-term (amino acids 1–126) into rabbits. Anti-RGSH, anti-β-catenin, and anti-FLAG antibodies were purchased from Qiagen, Transduction Laboratories, and Sigma, respectively. For coimmunoprecipitation assays, HeLa cells were transfected with His-β-catenin or His-arm1–12 using LipofectAMINE. Cells were lysed in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, and protease inhibitor mixture. Cell lysates were incubated with polyclonal anti-FHL2 antibody. Bound proteins were eluted and analyzed by immunoblotting with anti-RGSH monoclonal antibodies at 1:1000 dilution.

Immunofluorescence staining was carried out as described previously (29). HeLa cells grown on coverslips were transfected with 2 μg of β-catenin T41A and FLAG–FHL2 with calcium phosphate. 24 h later, cells were washed in phosphate-buffered saline, fixed with 3.7% paraformaldehyde, and permeabilized with 0.5% Triton X-100 in phosphate-buffered saline. Cells were then incubated with monoclonal mouse anti-β-catenin and polyclonal rabbit anti-FLAG antibodies followed by incubation with the corresponding Texas Red- and fluorescein isothiocyanate-coupled secondary antibodies. Images were obtained on a Leica DMRB microscope equipped with a Princeton CoolSnapFx CCD camera controlled by MetaVue software.

RT-PCR Analysis—Frozen tumor tissues were obtained after surgery from different French hospitals. Total RNA was isolated from tumor and liver tissues using RNA-Plus RNA extraction solution (Quantum
Interaction of FHL2 with β-Catenin

RESULTS

Interaction of FHL2 with β-Catenin—To identify β-catenin-binding proteins, a yeast two-hybrid system was used to screen a HeLa cDNA expression library with β-catenin arm repeats 1–10 (amino acids 132–554) as a bait (Fig. 1A). One clone interacting specifically with β-catenin contained the coding sequence for full-length FHL2 (279 amino acids) (Fig. 1B). The interaction of FHL2 with β-catenin was further confirmed by GST pull down. Using GST-β-catenin bound to Sepharose beads and in vitro expressed 35S-labeled FHL2 or conversely with GST-FHL2 and 35S-labeled β-catenin, we found specific binding of FHL2 to β-catenin, since GST alone was not able to pull down the binding partners (Fig. 2A).

FHL2 and β-catenin interaction was tested next by coimmunoprecipitation experiments. We generated a polyclonal antibody against the N-terminal domain of FHL2, which recognized specifically the FHL2 protein (Fig. 2B). His-tagged full-length β-catenin and His-tagged arm repeats 1–12 were transiently transfected in HeLa cells, and cell lysates were precipitated by FHL2 antibody followed by immunoblotting analysis with anti-His antibody. Both full-length and arm1–12 β-catenin proteins were revealed in the immune complexes (Fig. 2C, panel I). Specific coimmunoprecipitation of β-catenin with FHL2 was verified using preimmune sera that failed to precipitate β-catenin (Fig. 2C, panel IV). Thus, the interaction of FHL2 with β-catenin observed in the two-hybrid system also occurs in mammalian cells.

To test the binding specificity between β-catenin and FHL2, we next tested whether ACT, a related member of the FHL family (30), can interact with β-catenin in the yeast two-hybrid assay. When full-length ACT in the pACT2 vector was cotransformed with the β-catenin bait arm1–10 into yeast cells, no interaction was observed between β-catenin and ACT (data not shown).

Mapping of Interaction Domains in FHL2 and β-Catenin—Serial deletion mutants of β-catenin carrying various arm repeat sequences in-frame with GAL4 DBD (Fig. 1A) were first tested for their trans-activation activity in yeast, and constructs devoid of intrinsic activity were used to map the region mediating the binding to FHL2. Stable expression of β-catenin fragments in yeast was confirmed by immunoblotting with anti-GAL4 antibody (data not shown). Our results show that the arm repeats 3–8, which mediate β-catenin binding to LEF1 (31), were required but not sufficient for interaction with FHL2 (see Fig. 1A). Deletion of arm repeat 10 resulted in a 4-fold increase of binding activity, designating β-catenin arm repeats 1–9 as the optimal domain responsible for FHL2 binding.

To map the FHL2 domain mediating the interaction with β-catenin, overlapping constructs containing different LIM domains in fusion with GAL4 activation domain were tested for interaction with β-catenin arm repeats 1–10 in the yeast (see Fig. 1B). Although truncated FHL2 proteins were stably expressed in yeast as revealed by immunoblotting with anti-His antibody (panel I). Expression of β-catenin (panel II) and FHL2 (panel III) in HeLa cells was revealed by immunoblotting with His and FHL2 antibodies. The arrow in panel I indicates His-tagged arm repeats 1–12 immunoprecipitated by FHL2. IP, immunoprecipitation; IB, immunoblotting.
pressed in yeast (data not shown), all of the four and half of LIM domains were required for interaction with \(\beta\)-catenin, consistent with the finding that only full-length FHL2 was pulled out from the initial two-hybrid screen.

**FHL2 Stimulates \(\beta\)-Catenin-activated Transcription from TCF-responsive Promoters**—The \(\beta\)-catenin-TCF complex activates gene expression in response to Wnt signaling. Therefore, we assessed the effect of FHL2 on \(\beta\)-catenin trans-activating function in 293 cells using the TOPFLASH luciferase reporter, which contains TCF/LEF consensus-binding sites. As expected, the TOPFLASH reporter gene activity was enhanced by 20-fold by the constitutively active \(\beta\)-catenin T41A in which threonine 41 was changed to alanine, as frequently found in tumors (Fig. 3A) (32). Importantly, when FHL2 was coexpressed with \(\beta\)-catenin, the reporter gene activity was further enhanced up to 4.5-fold in a dose-dependent manner (Fig. 3A), indicating synergistic cooperation between FHL2 and \(\beta\)-catenin on trans-activation of the reporter gene. FHL2 trans-activation activity was dependent on \(\beta\)-catenin, because expression of the dominant negative TCF4 (\(\Delta\)NTCF4), which retains DNA binding activity but fails to interact with \(\beta\)-catenin, completely abolished this effect (Fig. 3A). Furthermore, coexpression of FHL2

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**Fig. 3. TCF/LEF-dependent activation of \(\beta\)-catenin by FHL2.** A, luciferase reporter assay with TOPFLASH/FOPFLASH. Plasmid amounts are in micrograms. Luciferase activity in 293 cells transfected with the TOPFLASH reporter and empty vector was arbitrarily determined as 1. B, luciferase assay with TOPFLASH/FOPFLASH reporters in SW480 cells. The ratio of luciferase activity in cells transfected with TOPFLASH versus FOPFLASH reporters is shown. C and D, luciferase assays with a reporter gene under control of the cyclin D1 promoter (C) and the IL-8 promoter (D) in 293 cells. The wild type IL-8 promoter (193-IL8) as well as the promoter with mutated Tcf site (193mt-IL8) was used in the assay. E, subcellular localization of transfected \(\beta\)-catenin and FHL2 in HeLa cells. Cells cultured on coverslips were transfected with \(\beta\)-catenin T41A and FLAG-FHL2. 24 h after transfection, cells were immunostained with monoclonal anti-\(\beta\)-catenin and polyclonal anti-FLAG antibodies followed by Texas Red-conjugated anti-mouse and fluorescein isothiocyanate-conjugated anti-rabbit secondary antibodies.
that FHL2 expression might play a role in tumor cells by compared with matched nontumor livers. This finding suggests of the tumor samples (Fig. 4). Importantly, in 8 of 10 cases, cases (32, 33). FHL2 expression was detected by RT-PCR in all

flash), demonstrating that the synergistic function of FHL2 and β-catenin was dependent on TCF/LEF. However, FHL2 alone had a weak dose-dependent effect on TOPFLASH and FOPFLASH reporter gene activity (Fig. 3A, lane 10), suggesting that it might also activate transcription through β-catenin-independent mechanisms.

In SW480 colon carcinoma cells, endogenous wild type β-catenin is constitutively active because of defective adenomatous polyposis coli in this cell line. Transient expression of FHL2 in SW480 cells resulted in dose-dependent activation of the TOPFLASH reporter, which was inhibited by the dominant negative Tcf4 (Fig. 3B). Thus, FHL2 is able to stimulate the trans-activating activity of both wild type and stabilized mutant β-catenin.

We then assessed the synergistic function of FHL2 and β-catenin on natural TCF-responsive promoters. 293 cells were transfected with a luciferase reporter controlled by either the cyclin D1 promoter or the IL-8 promoter, recently identified as a direct β-catenin-TCF target (11). As found for the synthetic TOPFLASH reporter, FHL2 increased the cyclin D1 promoter activity in association with β-catenin in a dose-dependent manner (Fig. 3C). This cooperative effect was strongly inhibited by ΔNTcf4 and totally abolished in reporter assays using a cyclin D1 promoter carrying mutated TCF-binding sites (data not shown). Similarly, coexpression of β-catenin and FHL2 synergistically activated the wild type IL-8 promoter, and this effect was abolished when the TCF-binding site in the promoter was mutated (Fig. 3D). These data suggest that the synergistic interaction of FHL2 and β-catenin might function in vivo on Wnt-responsive genes.

We next examined cellular localization of β-catenin and FHL2 in transfected cells by immunofluorescence analysis. When constitutively active β-catenin T41A and FLAG-tagged FHL2 were exogenously expressed in HeLa cells, the two proteins showed a predominant nuclear staining (Fig. 3E), suggesting that their interaction may occur in the nucleus.

Increased FHL2 Expression in Liver Tumors—To assess the implication of FHL2 in activated expression of cancer-related Wnt-responsive genes, we examined its expression in hepatoblastoma in which a high rate of genetic mutations is associated with nuclear accumulation of β-catenin in a majority of cases (32, 33). FHL2 expression was detected by RT-PCR in all of the tumor samples (Fig. 4). Importantly, in 8 of 10 cases, FHL2 expression was markedly up-regulated in tumors compared with matched nontumor livers. This finding suggests that FHL2 expression might play a role in tumor cells by enhancing the trans-activation function of β-catenin.

Enhancement of AR Transcriptional Activity by FHL2 and β-Catenin—It has been shown that FHL2 as well as β-catenin can individually interact with AR and activate AR-driven transcription (23, 24). The binding of FHL2 to AR was confirmed by GST pull-down experiments using GST-FHL2 and in vitro translated 35S-labeled AR (Fig. 5A). To explore the potential effect of the interaction between FHL2 and β-catenin coactivators on AR function, we cotransfected CV1 cells with a luciferase reporter gene under control of the mouse mammary tumor virus promoter (pMMTV-Luc) known to be regulated by steroid hormone receptors and vectors expressing wild type β-catenin, FHL2, and human AR. In accordance with previous reports, luciferase activity was enhanced ~2-fold by either β-catenin or FHL2 in the presence of DHT (Fig. 5B). When β-catenin and FHL2 were coexpressed with AR, they enhanced AR transcriptional activity by ~4-fold (Fig. 5B), indicating that the combined effect of both proteins was only additive. This effect was dependent on the presence of DHT, indicating that transcriptional activation of AR by β-catenin and FHL2 was not attributed to nonspecific interactions of β-catenin or FHL2 with the reporter. This result was further confirmed by the observation that β-catenin and FHL2 were not able to activate the MMTV promoter in the absence of AR (Fig. 5B).

DISCUSSION

The association of β-catenin with TCF is an essential step in the transduction of the Wnt signal, and transcriptional activity of the β-catenin-TCF complex can be modulated by coactivators and corepressors that interact with β-catenin or TCF. Here we report that FHL2 is a novel partner and coactivator of β-catenin. The physical interaction between FHL2 and β-catenin was demonstrated by in vitro and in vivo assays, including yeast two-hybrid screens, in vitro pull-down assays, and coinmunoprecipitation. Further characterization of the interaction using the yeast two-hybrid assay showed that β-catenin arm repeats 1–9 and the complete set of LIM domains in FHL2 were required for optimal binding. By contrast, no interaction could be detected between β-catenin and ACT, a LIM domain protein closely related to FHL2, ruling out the possibility that LIM domains might mediate nonspecific association with β-catenin. Furthermore, we show that FHL2 potentiates β-catenin transactivating function on TCF/LEF-dependent transcription of
Wnt-responsive genes such as *cyc1 D1* and interleukin-8 in human kidney and colon cells. Importantly, FHL2 expression in the absence of nuclear β-catenin did not affect the activity of TCF target gene promoters, and cooperation between FHL2 and β-catenin was strictly dependent upon the presence of consensual TCF-binding sites in the cyc1 D1 and IL-8 promoters. Finally, ectopically expressed FHL2 and β-catenin were found to colocalize in HeLa cell nuclei, suggesting that nuclear interaction of these proteins might be involved in enhancing TCF transcription.

Taken together, our data support the idea that FHL2 might be recruited by β-catenin to TCF-dependent promoters, although no direct evidence has been provided so far for the interaction of FHL2 at an endogenous β-catenin target promoters. While this paper was in revision, similar conclusions were reported by Martin et al. (34) who demonstrated that FHL2 specifically and functionally interacts with endogenous β-catenin in *vivo* but not with LEF-1 and that β-catenin is able to bind simultaneously FHL2 and LEF-1, forming a ternary protein complex in *vitro*. The authors identified the N-terminal region and first arm repeat of β-catenin as the FHL2-binding region. However, in our study, the arm repeats 1 and 2 of β-catenin were required for the interaction with FHL2, but optimal binding efficiency was observed for repeats 1–9. This apparent discrepancy might be explained by different strategies that have been used to localize the interacting regions.

Alternatively, it is conceivable that different β-catenin domains might be able to bind FHL2. It is striking that different regions in β-catenine have been implicated in the binding of several partners, including TATA-binding protein (12), CBP/p300 (13–15, 35), and adenomysis polyposis coli (36, 37).

In previous studies, FHL2 has been shown to bind different transcription factors acting either as a transcriptional coactivator or as a corepressor (21, 23, 38). Interestingly, FHL2 has been shown to activate β-catenin-dependent transcription in epithelial cells (this study and Ref. 34) while it has opposite down-regulating effect in myoblasts, suggesting a cell type-specific regulation of β-catenin function by FHL2 (34). LIM domains function as molecular adapters mediating the assembly of multicomponent complexes. Therefore, FHL2 might target other cofactors to β-catenin to link the β-catenin-TCF complex to the RNA polymerase II machinery or to chromatin-remodeling complexes.

The impact of FHL2-β-catenin interaction on AR transcriptional activity remains unclear, since we did not observe a cooperative effect of these AR coactivators. Furthermore, all of the FHL2 deletion mutants analyzed had lost the ability to bind AR as well as β-catenin, and the activity of FHL2 and β-catenin on AR transcriptional function did not appear to be dose-dependent.2 Therefore, our data might be interpreted as additive effects of each individual protein in contrast with the results obtained for TCF/LEF, which indicates that synergetic activity of β-catenin and FHL2 may be dependent on the promoter context. Further studies using FHL2 null cells or FHL2 mutants retaining only binding to either AR or β-catenin would shed light on the role of FHL2-β-catenin interaction on AR function.

Whether the interaction of FHL2 with β-catenin plays a role during oncogenesis is an interesting issue. In human cancers affecting epithelial tissues, β-catenin is frequently activated and the stimulating function of FHL2 on β-catenin might have impact on oncogenic processes. This notion is strongly supported by our finding that the *cyc1 D1* promoter is markedly activated by FHL2 in a β-catenin-dependent manner and that FHL2 expression is up-regulated in hepatoblastoma. In this pediatric liver tumor, β-catenin was found to be mutated in >50% of cases (32, 33), and overexpression of *cyc1 D1* was correlated with β-catenin mutation (39). In recent studies of prostate cancer (40), nuclear expression of FHL2 has been detected at higher levels in tumor cells than in normal prostate epithelium, and nuclear trans-location and transcriptional activity of FHL2 might be regulated by Rho signaling. Rho family members are frequently overexpressed in human tumors, and the activation of Rho signaling might be involved in the migration and dissemination of tumor cells. Localized in both cytoplasm and nucleus, FHL2 has been found to bind multiple partners including α- and β-integrins that are key regulators of cell adhesion and migration (22). Thus, functional interactions between FHL2 and β-catenin might have important consequences in multiples aspects of the activities of both proteins.

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