MOLECULAR AND CELLULAR BIOLOGY, Oct. 2009, p. 5477–5487 Vol. 29, No. 20
0270-7306/09/$08.00+0 doi:10.1128/MCB.00335-09

Nuclear-Cytoplasmic Shuttling of Menin Regulates Nuclear Translocation of β-Catenin▽

Yanan Cao,[1]† Ruixin Liu,[1]† Xiuli Jiang,[1] Jieli Lu,[1] Jingjing Jiang,[1] Changxian Zhang,[3] Xiaoying Li,[1,4]* and Guang Ning[1,2]*

Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrinology and Metabolism and Chinese-French Laboratory of Genomics and Life Sciences, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, 197 Ruijin 2nd Road, Shanghai 200025, China[1]; Laboratory for Endocrine and Metabolic Diseases, Institute of Health Sciences, SIBS, Chinese Academy of Sciences, China[2]; Laboratoire Genetique et Cancer, CNRS, UMR5201, Faculte de Medecine Univ-Lyon1, 69008 Lyon, France[3]; and Shanghai Key Laboratory for Endocrine Tumors, E-Institute of Shanghai Universities, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China[4]

Received 16 March 2009/Returned for modification 8 April 2009/Accepted 8 July 2009

Menin, which is encoded by the multiple endocrine neoplasia type 1 (MEN1) gene, is a tumor suppressor and transcriptional regulator. Menin controls proliferation and apoptosis of cells, especially pancreatic β cells. We have found that menin contains two functional nuclear export signals and that there is nuclear accumulation of β-catenin in *Men1*-null mouse embryonic fibroblasts and insulinoma tissues from β-cell-specific *Men1* knockout mice. It is reported that the deregulation of Wnt/β-catenin signaling caused by inactivation of tumor suppressors results in abnormal development or tumorigenesis. We further revealed that overexpression of menin reduces β-catenin nuclear accumulation and its transcriptional activity. Menin is able to directly interact with β-catenin and carry β-catenin out of the nucleus via nuclear-cytoplasmic shuttling in a CRM1-dependent manner. These results imply that menin may control cell proliferation through suppression of Wnt/β-catenin signaling.

Pancreatic islet tumors, including insulinoma and gastrinoma, usually occur in multiple endocrine neoplasia type 1 (MEN1) patients. *Men1* codes for menin, a 610-amino-acid protein. Heterozygous *Men1* mutations result in loss of heterozygosity and complete loss of menin in tumor tissues (5, 12, 24). Menin, containing three nuclear localization signals (NLS), is recognized as a nuclear protein (12, 23). Previous studies have demonstrated that menin participates in the regulation of gene transcription, cell proliferation, and apoptosis. Menin interacts with a number of transcription factors, including JunD, NF-κB, and Smad3 (1, 15, 18), and, in association with histone methyltransferases MLL1 and MLL2, regulates some crucial gene expression, including expression of the cyclin-dependent kinase inhibitor genes $p18^{INK4c}$ and $p27^{Kip1}$ (19, 28, 45). However, the role of menin as a tumor suppressor in MEN1 tumor development remains to be elucidated. Menin has been demonstrated to participate in islet β-cell proliferation. Deletion of *Men1* accelerates cell proliferation and cell cycle progression (19, 37). Expression of menin in maternal β cells of pregnant mice negatively regulates pancreatic islet expansion and insulin production, which is closely related to gestational diabetes mellitus (20). However, these novel findings raise a crucial question about the key partners of menin in β cells and the molecular mechanism of menin involvement in β-cell proliferation. It was reported that β-catenin was reduced in cytoplasmic membranes and accumulated in cytoplasm in advanced insulinoma in β-cell-specific *Men1* knockout mice (4). Membrane E-cadherin of pretumor β cells is reduced in the absence of menin (44). These observations indicate that the canonical Wnt/β-catenin pathway may be activated in menin-null β cells. Nevertheless, it is also reported that menin promotes the activity of the Wnt signaling pathway by regulation of axin 2 expression (6).

The Wnt/β-catenin signaling controls multiple developmental processes and is associated with various malignancies. In response to canonical Wnt signaling, the stabilized and nucleus-accumulated β-catenin differentially regulates downstream target genes through interaction with T-cell-specific factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factors (7, 26). It has been demonstrated that some tumor suppressors, including adenomatous polyposis coli (APC), PTEN, and WTX, regulate β-catenin degradation or nuclear export. The inactivation of these tumor suppressors leads to deregulation of Wnt/β-catenin signaling and results in tumorigenesis, including colon cancer and Wilms' tumor (27, 29, 32).

Recent studies demonstrate that Wnt/β-catenin signaling regulates the organogenesis and development of the pancreas and participates in islet β-cell proliferation, indicating a key role of Wnt signaling in diabetes (17, 30, 42). In transgenic mouse models, the activation of canonical Wnt/β-catenin signaling promotes an increase of β-cell mass and insulin secretion, whereas its inactivation impairs β-cell expansion and glucose tolerance (36). Expression of stabilized β-catenin at a late stage of pancreas development promotes growth of mouse

* Corresponding author. Mailing address: Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, 197 Ruijin 2nd Road, Shanghai 200025, China. Phone: 86 21 64370045. Fax: 86 21 64373514. E-mail for Xiaoying Li: lixy@sibs.ac.cn. E-mail for Guang Ning: guangning@medmail.com.cn.
† These authors contributed equally to this work.
▽ Published ahead of print on 3 August 2009.

pancreas and leads to nuclear β-catenin accumulation in abnormal islets (13). Overexpression of active glycogen synthase kinase 3β (GSK3β), an inhibitor of Wnt signaling, impairs glucose tolerance and decreases β-cell mass in mice (25). β-Cell replication can be increased by treatment with diverse GSK3 inhibitors (31). However, a recent study has reported that Wnt signaling inhibits β-cell proliferation and that a GSK3β inhibitor reduces β-cell replication (6). Although some contradictory results have been reported, these studies have shed light on the precise function and regulation of Wnt/β-catenin signaling in pancreatic development and tumorigenesis.

In the present study, we demonstrate that menin contains two functional nuclear export signals (NES) and shuttles between the cytoplasm and nucleus, regulating subcellular localization of β-catenin via nuclear export function. The absence of menin leads to nuclear β-catenin accumulation and transcriptional activation of the target genes. We speculate that nuclear β-catenin accumulation coupled with loss of menin is associated with pancreatic β-cell proliferation and tumorigenesis.

MATERIALS AND METHODS

Mice. β-Cell-specific *Men1* knockout mice were generated previously (3, 4). All animal experiments were conducted in accordance with accepted standards of humane animal care and were approved by the regional animal ethics committee of CNRS Rhône-Auvergne.

Plasmids and reagents. All *Men1* expression constructs were created with standard PCR-based cloning strategies. Human full-length *Men1* cDNA was inserted in pCI-neo vector (Promega, Madison, WI), pCMVFa vector, or the pEGFP-N2 and pEGFP-C1 vectors (Clontech, Palo Alto, CA). Mutations were introduced into plasmids with the QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instruction. Plasmids encoding green fluorescent protein (GFP)–β-catenin and β-catenin* (stable β-catenin with a Ser-to-Ala mutation in the GSK3 phosphorylation site), tagged with KT3 or GFP (2, 46), were obtained from Xiang Yu (Institute of Neuroscience, SIBS, CAS, Shanghai) with permission from James Nelson (Stanford University). Potential NES sequences of menin or human immunodeficiency virus (HIV) Rev were inserted into pEGFP-C1 vectors with appropriate restriction enzymes. The oligonucleotide sequences are as follow: HIV Rev (amino acids [aa] 75 to 83), 5′-TCGACGCTCTACCACCGCTTGAGAGACTTACTC TTG-3′; *Men1* NES1 (aa 34 to 41), 5′-TCGACGACCTGGTGCTCCTTTCCT TGGTGCTGG-3′; NES2 (aa 254 to 259), 5′-TCGACTCGCTGGAGCTTCTG CAGCTGG-3′; and NES3 (aa 259 to 267), 5′-TCGACCAGCTGCAGCAGAA GCTGCTCTGGCTGCTCG-3′. Three pairs of *Men1* small interfering RNA (siRNA) oligonucleotide sequences were as follows: 5′-GGGUAGUGUUUGG GCCCAATT-3′ and 5′-UUGGGCCCAAACACUACCCAG-3′; 5′-GAAGGU CUCCGAUGUCAUATT-3′ and 5′-UAUGACAUCGGAGACCUUCTT-3′; and 5′-GGGAAGACGAGGAGAUCUATT-3′ and 5′-UAGAUCUCCUCGU CUUCCCGG-3′. Nonspecific control siRNA oligonucleotide sequences were 5′-UUCUCCGAACGUGUCACGUTT-3′ and 5′-ACGUGACACGUUCGGA GAATT-3′. Leptomycin B (LMB), cycloheximide (CHX), MG132, and polyethylene glycol 3350 were purchased from Sigma (St. Louis, MO).

Cell culture. Human embryonic kidney 293T cells, HeLa cells, and mouse embryonic fibroblasts (MEFs) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, CA) at 37°C in a 5% CO_2–95% air atmosphere. Cells were transfected using Lipofectamine 2000 or Lipofectamine LTX (Invitrogen) according to the manufacturer's instructions.

Protein preparation and Western blots. To prepare total protein extracts, cells were washed with phosphate-buffered saline and lysed in radioimmunoprecipitation assay buffer containing 50 mM Tris-HCl (pH 8), 150 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 1 mM NaF, 1% NP-40, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail and phosphatase inhibitor cocktail (Sigma, St. Louis, MO). Nuclear and cytoplasmic lysates were prepared with the NE-PER kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. Equal amounts of proteins were separated on 7.5% polyacrylamide-sodium dodecyl sulfate gels and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 10% nonfat milk in Tris-buffered saline with Tween for 2 h and then incubated with the following primary antibodies: polyclonal anti-β-catenin (1:1,000; Cell Signaling), polyclonal anti-α-tubulin (1:1,000; Cell Signaling), polyclonal anti-Hsp70 (1:1,000; Cell Signaling), polyclonal antimenin (1:2,000; Bethyl Laboratories), monoclonal anti-GFP (1:2,000; Sigma, St. Louis, MO), monoclonal anti-β-actin (1:60,000; Calbiochem), polyclonal anti-lamin B (1:1,000; Santa Cruz Biotechnology). Membranes were then washed with Tris-buffered saline with Tween and incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling) for 1 h. The proteins were visualized with enhanced chemiluminescence reagents according to the manufacturer's protocol (Amersham Pharmacia, Little Chalfont, Buckinghamshire, England).

Coimmunoprecipitation and in vitro binding assay. Whole-cell lysates of 293T cells coexpressing Flag-menin and GFP–β-catenin were used to perform coimmunoprecipitation experiments according to the standard protocol. Glutathione *S*-transferase (GST) pulldown assays were performed following the protocols previously described (16). Normal mouse and rabbit immunoglobulin Gs (Sigma) were used as the negative controls.

Luciferase reporter assays. TOP-flash and FOP-flash (21) were obtained from Xiang Yu, with permission from Hans Clevers. pRL-TK, expressing *Renilla* luciferase, was purchased from Promega (Madison, WI). 293T cells were seeded at 3×10^5 cells per well in 12-well plates and transfected with 200 ng or 400 ng PCI-Men1 or PCI-antisense-Men1 (control) plasmids together with 35 ng of reporter, 35 ng of GFP–β-catenin, and 3.5 ng of pRL-TK. Cells were harvested at 24 h after transfection, and luciferase activities measured. Values were normalized using the dual-luciferase reporter assay system (Promega).

Immunofluorescence and immunohistochemical staining. Cells grown on poly-L-lysine (Sigma)-pretreated coverslips or eight-well chamber slides were fixed with 4% paraformaldehyde or 100% cold acetone. Insulinoma tissues obtained from MEN1 patients during surgery were deparaffinized by standard techniques. Immunofluorescence and immunohistochemical staining were performed according to the standard protocol. The following primary antibodies were used in this study: polyclonal rabbit antimenin antibody (1:1,000; Bethyl Laboratories), goat antimenin antibody (1:200; Santa Cruz Biotechnology), and anti-β-catenin antibody (1:100; Cell Signaling) and monoclonal anti-β-catenin antibody (Sigma) and anti-β-actin (1:10,000; Calbiochem). The secondary antibodies for immunofluorescence staining were purchased from Invitrogen. The images were acquired using a Zeiss confocal microscopy or an Olympus system.

Heterokaryon formation assay. The heterokaryon assays were carried out according to published procedures (33, 39). Briefly, HeLa cells were transfected with plasmids expressing menin-GFP. At 48 hours after transfection, $Men1^{-/-}$ MEFs were added to the culture. The cells were treated with 100 μg/ml CHX (Sigma) for 0.5 h before they were fused by treatment with 50% polyethylene glycol 3350–phosphate-buffered saline. After another 3 h of incubation in the presence of CHX (100 μg/ml), the cells were fixed to perform immunofluorescence staining. For the LMB group, cells were treated with 50 ng/ml LMB for 3 h before and 3 h after the fusion.

FRAP. Fluorescence recovery after photobleaching (FRAP) analysis was performed according to published procedures (22). Briefly, live $Men1^{-/-}$ MEFs expressing GFP-menin were subjected to quantitative FRAP analysis. FRAP analysis was performed with a Nikon Eclipse Ti inverted microscope system. Cells were monitored using the laser at 488 nm and bleached at 100% laser power. An area within the cytoplasm was bleached with maximum laser power for 2 min. Subsequently, images were taken as follows: 30 frames at 1-s intervals and 21 frames at 30-s intervals. Fluorescence intensities in the bleached area and in the nuclear regions, as well as the background signal, were quantified. The raw data obtained for recovery were corrected for the background intensity. The ratio in the prebleach image was set to 100%. The recovery curves are representative of 20 cells in two independent experiments.

Real-time reverse transcription-PCR (RT-PCR). Quantitative PCR analysis was performed using MEF cDNA as a template on an Applied Biosystems 7300 real-time machine. The sequences of the primers used in this study are as follows: mouse β-catenin forward, 5′-CCCAGTCCTTCACGCAAGAG-3′; mouse β-catenin reverse, 5′-CATCTAGCGTCTCAGGGAACA-3′; mouse APC forward, 5′-CTTGTGGCCCAGTTAAAATCTGA-3′; mouse APC reverse, 5′-CG CTTTTGAGGGTTGATTCCT-3′; mouse GSK3β forward, 5′-TGGCAGCAA GGTAACCACAG-3′; mouse GSK3β reverse, 5′-CGGTTCTTAAATCGCTTG TCCTG-3′; mouse axin forward, 5′-ACTGAGGTATTAGGGTGCAGC-3′; mouse axin reverse, 5′-GCATCTTCGGTGAAACTTGCTC-3′.

Statistical analysis. All statistical analyses were performed using Student's *t* test. Error bars in graphs represent standard deviations (SD).

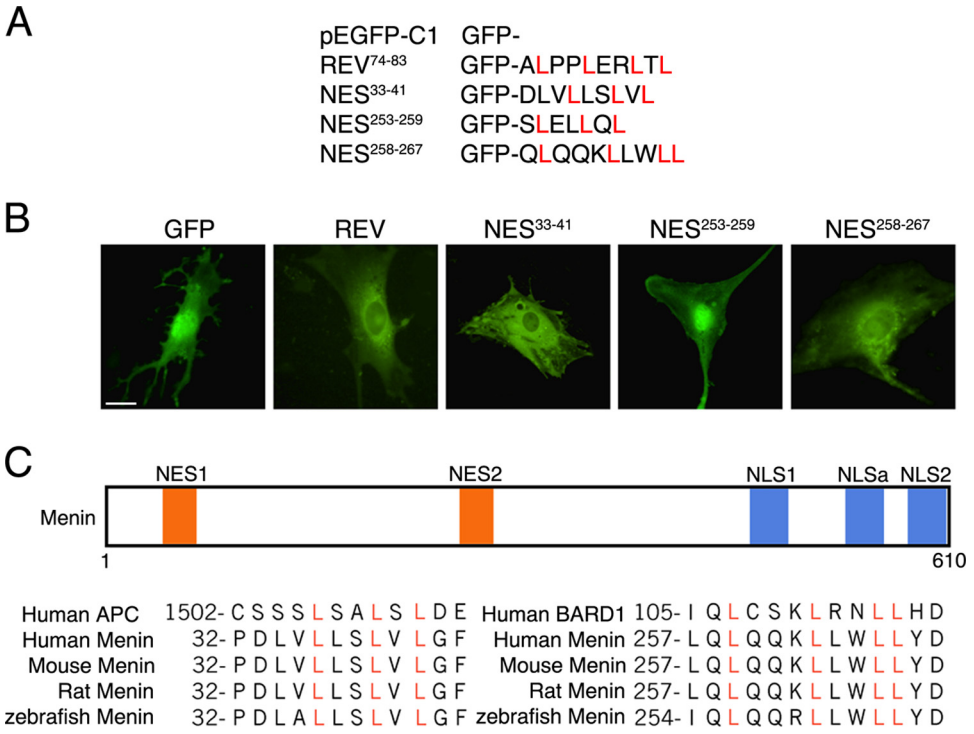
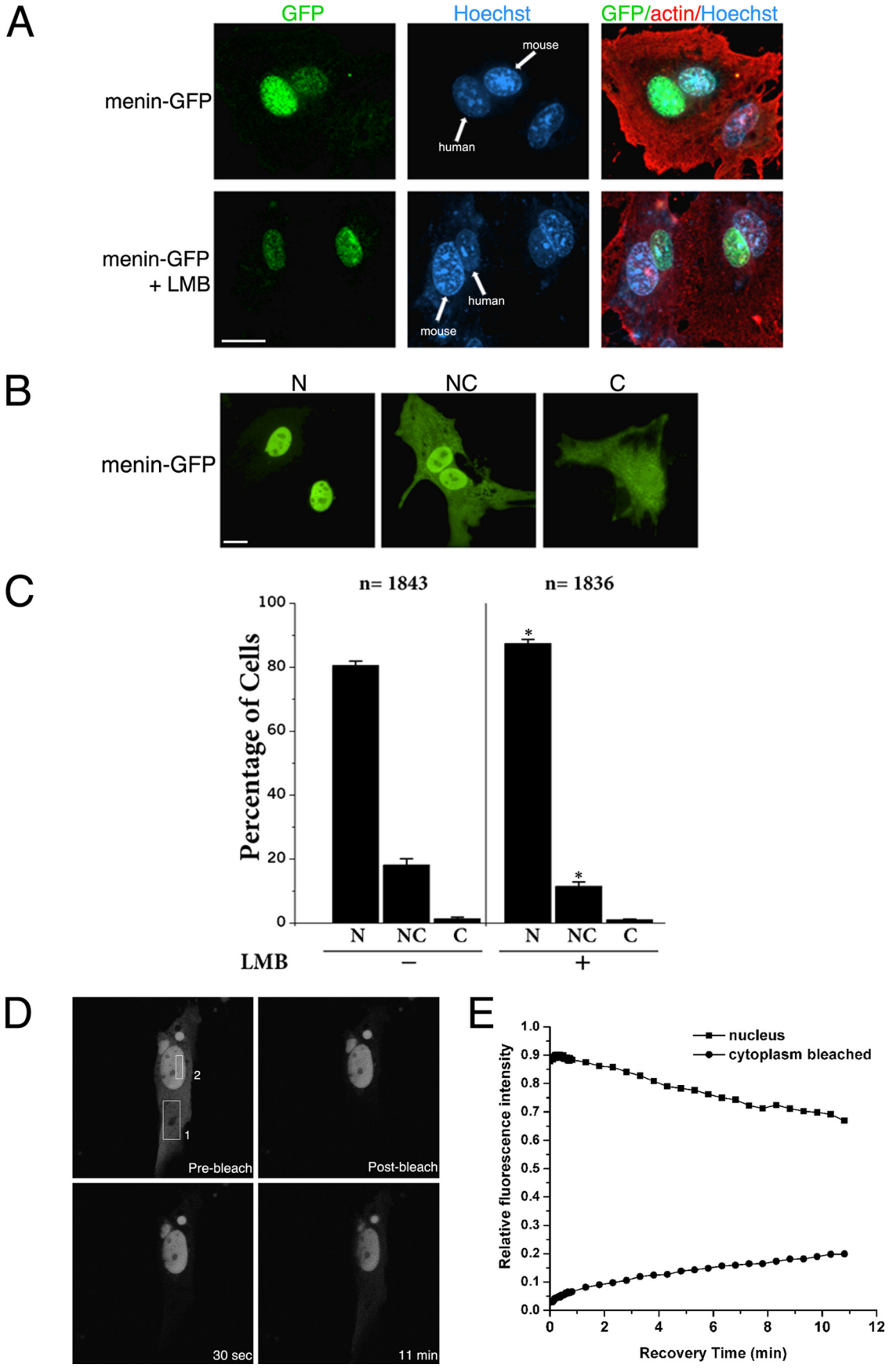


FIG. 1. Two functional NES identified in menin. (A) Potential NES sequences of menin ($NES^{33–41}$, $NES^{253–259}$, and $NES^{258–267}$) and HIV Rev ($REV^{74–83}$) sequences were inserted into pEGFP-C1 vectors to generate fusion proteins. (B) Three potential NES in menin (aa 36 to 41, 254 to 259, and 258–267) were identified, inserted into the pEGFP-C1 vector, and transfected into *Men1*$^{-/-}$ MEFs. The GFP fluorescence (green) was observed at 24 h after transfection. A HIV Rev NES was used as a positive control. NES1 (aa 36 to 41) and NES2 (aa 258 to 267) were functional in export from the nucleus. (C) Sequence conservation of menin NES. NES1 (aa 36 to 41) is located in the N terminus and NES2 (aa 258 to 267) in the middle of menin. NLS1, NLS2, and NLSa are located in the C terminus of menin, as reported previously (20, 45). Both NES1 and NES2 are highly conserved in human, mouse, rat, and zebrafish.

RESULTS

Menin contains functional NES and shuttles between cytoplasm and nucleus. Previous studies showed that several tumor suppressors, which contain both of NLS and NES, shuttles between the cytoplasm and nucleus through the nuclear pore complex (11). Nuclear-cytoplasmic shuttling of some tumor suppressors, such as APC and axin, could bind and export β-catenin to the cytoplasm for degradation or cell adhesion (8, 14, 35). We speculated that menin, a nuclear protein, could also shift to the cytoplasm. It has been demonstrated that menin contains three NLS (NLS1, NLS2, and NLSa) domains in the C terminus (12, 23). We analyzed menin sequences and identified three potential NES sites in two leucine zipper-like motifs. The sequence characteristics of the first (aa 33 to 41) and the second (253 to 259 aa) NES sites are similar to those of APC (22), and those of the third (aa 258 to 267) are similar to those of BRAD1 (34). The second and the third NES sites share the same leucine (aa 259), forming two neighbor NES sites. Three NES sequences were synthesized and inserted into pEGFP-C1 vectors to generate GFP and NES fusion proteins (GFP-$NES^{33–41}$, GFP-$NES^{253–259}$, and GFP-$NES^{258–267}$), which were expressed in *Men1*$^{-/-}$ MEFs (Fig. 1A). Vehicle vectors and HIV Rev constructs were used as negative and positive controls as described previously (41). Live transfected cells were observed under a fluorescence microscope. GFP-$NES^{33–41}$ and GFP-$NES^{258–267}$ fusion proteins were localized mainly in the cytoplasm, like GFP-Rev. In contrast, GFP-$NES^{253–259}$ was located mainly in the nucleus (Fig. 1B). It was clearly shown that the first and the third NES sites ($NES^{33–41}$ and $NES^{258–267}$) were functional for nuclear export. We named these two functional NES sequences (LLSLVL and LQQKLL WLL) NES1 and NES2, which were well conserved in human, mouse, rat, and zebrafish (Fig. 1C).

To further validate the nuclear-cytoplasmic shuttling of menin, heterokaryon formation assays were performed. Human HeLa cells expressing menin-GFP were fused to mouse *Men1*$^{-/-}$ MEFs to observe the subcellular localization of menin. In 3 h of cell fusion, nuclear accumulation of menin in mouse nuclei of heterokaryons was observed, which indicated that menin migrated from human nuclei to mouse nuclei. In addition, we treated the cells with the CRM1-specific nuclear export inhibitor LMB and found that the nuclear translocation of menin in heterokaryons was blocked (Fig. 2A). To further explore menin shuttling in intact cells, we overexpressed menin in *Men1*$^{-/-}$ MEFs with or without LMB treatment to examine the distribution of menin (Fig. 2B). The menin distribution pattern was classified as nucleus (N), both nucleus and cytoplasm (NC), or cytoplasm (C). N pattern cells were significantly increased with LMB treatment (87.4% versus 80.5%; $P = 0.022$), whereas the NC pattern cells were significantly reduced (11.5% versus 18.2%; $P = 0.038$) (Fig. 2C). This suggested that menin shuttled through the nuclear pore com-


A
GFP
Hoechst
GFP/actin/Hoechst
menin-GFP
menin-GFP
+ LMB
mouse
human
human
mouse
B
N
NC
C
menin-GFP
C
n= 1843
n= 1836
Percentage of Cells
100
80
60
40
20
0
N
NC
C
N
NC
C
LMB
–
+
D
2
1
Pre-bleach
Post-bleach
30 sec
11 min
E
nucleus
cytoplasm bleached
Relative fluorescence intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
12
Recovery Time (min)

plex-dependent nuclear export receptor CRM1. In addition, we performed FRAP experiments with GFP-tagged menin to analyze the nuclear export. We expressed GFP-menin in *Men*$^{-/-}$ MEFs, bleached an area in the cytoplasm, and monitored the fluorescence intensities in this region and in the nucleus. The fluorescence in the bleached area recovered 20% in 11 min, and the fluorescence dropped to 70% in the nucleus (Fig. 2D and E). These results indicated that menin shuttles between nucleus and cytoplasm.

Loss of menin leads to nuclear accumulation of β-catenin. To investigate whether the nuclear-cytoplasmic shuttling of menin leads to β-catenin subcellular redistribution like that of the tumor suppressor APC, we used β-cell-specific *Men1* knockout mice to examine β-catenin localization in pancreatic tumors, which were generated as described previously (4). The pancreatic islets developed into early-stage insulinoma at the age of 6 months in *Men1* knockout mice. Immunofluorescence staining demonstrated that β-catenin accumulated mostly in the nuclei of menin-negative β cells in *Men1*-null mice (*Men1*$^{F/F}$-*RipCre*$^{+}$), compared with a major cytoplasmic membrane distribution of β-catenin in the control mice (*Men1*$^{F/F}$-*RipCr*$^{-}$) (Fig. 3A). The β-catenin redistribution was also observed in glucagonoma cells developed in *Men1* knockout mice (data not shown). These data indicate that Wnt/β-catenin signaling may participate in pancreatic β-cell proliferation and insulinoma development in MEN1.

It was reported that the proliferation and cell cycle progression of MEFs were accelerated with the ablation of *Men1* (37). We examined the expression and localization of β-catenin in *Men1*$^{-/-}$ MEFs. Immunofluorescence staining showed that β-catenin accumulated markedly in the nucleus in *Men1*$^{-/-}$ MEFs, compared with cytoplasmic membrane localization in *Men1*$^{+/+}$ MEFs (Fig. 3B). We also confirmed the β-catenin redistribution in another two pairs of *Men1*$^{-/-}$ and *Men1*$^{+/+}$ MEFs (data not shown). Expression of exogenous menin-GFP could reverse the nuclear accumulation of β-catenin in *Men1*$^{-/-}$ MEFs (Fig. 3C).

Overexpression of menin reduces the nuclear accumulation of β-catenin. We overexpressed wild-type menin in *Men1*$^{-/-}$ MEFs and found that endogenous nuclear β-catenin was markedly reduced (Fig. 4A). Meanwhile, the whole-cell β-catenin protein levels were also slightly reduced with menin overexpression; however, β-catenin mRNA levels were not changed (Fig. 4C). Moreover, we did not find any difference in whole-cell β-catenin between *Men1*$^{+/+}$ and *Men1*$^{-/-}$ MEF cells by Western blotting (Fig. 4B) and real-time PCR (Fig. 4C). Furthermore, overexpression of menin in *Men1*$^{-/-}$ MEFs did not significantly affect the expression of APC, axin, and GSK3β, which mediate β-catenin degradation in cytoplasm (Fig. 4D). In HeLa cells, knockdown of menin using *Men1*-specific siRNA dramatically increased endogenous nuclear and whole-cell β-catenin (Fig. 4E). We further coexpressed menin and stable-form β-catenin (GFP–β-catenin*) in HEK293T cells and found that overexpression of menin reduced only the nuclear instead of the whole-cell protein level of exogenous stable β-catenin (Fig. 4F). This implied that nuclear β-catenin reduction mediated by menin was not due to direct degradation. We used the TOP-flash and FOP-flash reporter system to measure β-catenin/LEF-mediated transcriptional activity in menin- and stable β-catenin-coexpressing 293T cells. Menin significantly inhibited β-catenin/LEF luciferase activities in a dose-dependent manner (Fig. 4G). These results indicate that menin reduces nuclear accumulation of β-catenin and suppresses β-catenin/LEF-mediated transcriptional activity independent of β-catenin degradation.

NES mutations impair nuclear export function and induce rapid degradation of menin. We further investigated whether menin exported nuclear β-catenin to the cytoplasm and facilitated β-catenin degradation. Menin or a vehicle vector control was coexpressed with β-catenin in 293T cells, which were further treated with LMB or mock treated. LMB treatment did not significantly change exogenous nuclear β-catenin levels (Fig. 5A), whereas nuclear and whole-cell β-catenin levels were increased by LMB treatment when menin and β-catenin were co-overexpressed (Fig. 5A). This indicated that nuclear β-catenin can be exported by menin in a CRM1-dependent way. We next explored the effects of NES-mutated menin on nuclear export of β-catenin. A series of menin-GFP vectors containing NES mutations were generated (Fig. 5B). Two key leucines in NES1 and NES2 were replaced by alanines individually (NES mut1 and NES mut2) or together (NES mut3). Western blotting showed that all three mutant NES menins failed to export nuclear β-catenin (Fig. 5C). In addition, two mutant menins derived from MEN1 patients, L267P and L264P, were generated. Leucine 267 was crucial for the NES2 domain, whereas leucine 264 was not. The nuclear export of β-catenin was impaired for the L267P mutant, whereas it was still maintained for the L264P mutant (Fig. 5D). Furthermore, mu-

FIG. 2. Nuclear-cytoplasmic shuttling of menin. (A) Heterokaryons of human HeLa cells expressing wild-type menin-GFP and mouse *Men1*$^{-/-}$ MEFs. The HeLa cells were transfected with wild-type menin-GFP, pretreated with CHX alone or with LMB and CHX at 24 h after transfection, and then fused to mouse *Men1*$^{-/-}$ MEFs. As heterokaryons were formed, the cells were incubated in the presence of CHX alone or LMB and CHX for an additional 3 h and then fixed and immunostained. In heterokaryons, GFP-menin (green) appeared in the nuclei of *Men1*$^{-/-}$ MEFs and was exported from the human HeLa cell nucleus. Human and mouse nuclei could be easily distinguished by different Hoechst staining patterns. Each image represents more than 50 heterokaryons analyzed on chamber slides. (B) Expression patterns of wild-type GFP-menin in *Men1*$^{-/-}$ MEFs. The GFP fluorescence (green) was observed under a microscope at 24 h after transfection. The cells were allocated into three patterns according to menin distribution, including nuclear localization (N), both nuclear and cytoplasmic localization (NC), and cytoplasmic localization alone (C). (C) Change of menin localization during treatment with LMB (50 ng/ml) or mock treatment for 6 h. The cells with the N pattern were significantly increased after LMB treatment compared with mock treatment (87.4% versus 80.5%; $P = 0.022$), and those with the NC pattern were significantly reduced (11.5% versus 18.2%; $P = 0.038$). The results obtained from three independent experiments are expressed as mean ± SD (*, $P < 0.05$). More than 1,800 cells were counted in each experiment. (D) FRAP assay. Live Men1$^{-/-}$ MEF cells expressing GFP-menin was subjected to FRAP analysis. A defined area in the cytoplasm (white rectangle 1) was bleached with maximum laser power for 2 min. The bleached region and a nuclear area (white rectangle 2) were monitored by imaging scan. (E) The fluorescence intensities of the bleached cytoplasmic area and the nuclear area were measured and then corrected for the background signal and plotted over time. Scale bar, 30 μm.

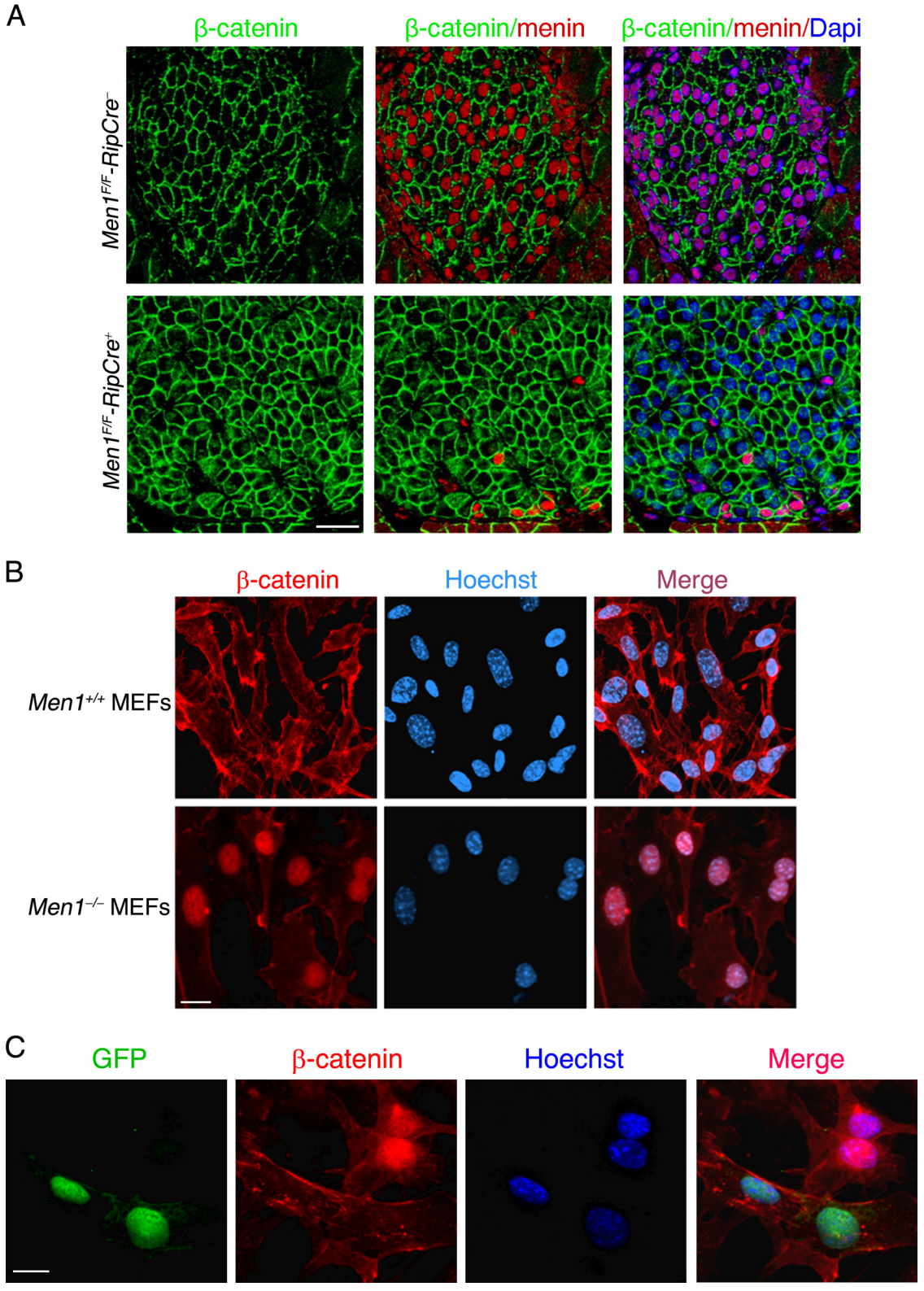


FIG. 3. Nuclear accumulation of β-catenin in the absence of menin. (A) Pancreatic islets obtained from 6-month-old *Men1*$^{F/F}$-*RipCre*$^{-}$ and *Men1*$^{F/F}$-*RipCre*$^{+}$ mice. Pancreas sections were stained with β-catenin antibody (green) and menin antibody (red). Nuclei were stained with DAPI (4′,6′-diamidino-2-phenylindole) (blue). (B) In menin-null MEFs (*Men1*$^{-/-}$), β-catenin (red) is largely accumulated in the nucleus, in contrast with cytoplasmic membrane localization in wild-type (*Men1*$^{+/+}$) MEFs. Nuclei are stained with Hoechst stain (blue). (C) Translocation of nuclear β-catenin. *Men1*$^{-/-}$ MEFs were transfected with menin-GFP. Cells were stained with β-catenin antibody (red). Nuclei are stained with Hoechst stain (blue). Scale bars, 30 μm.

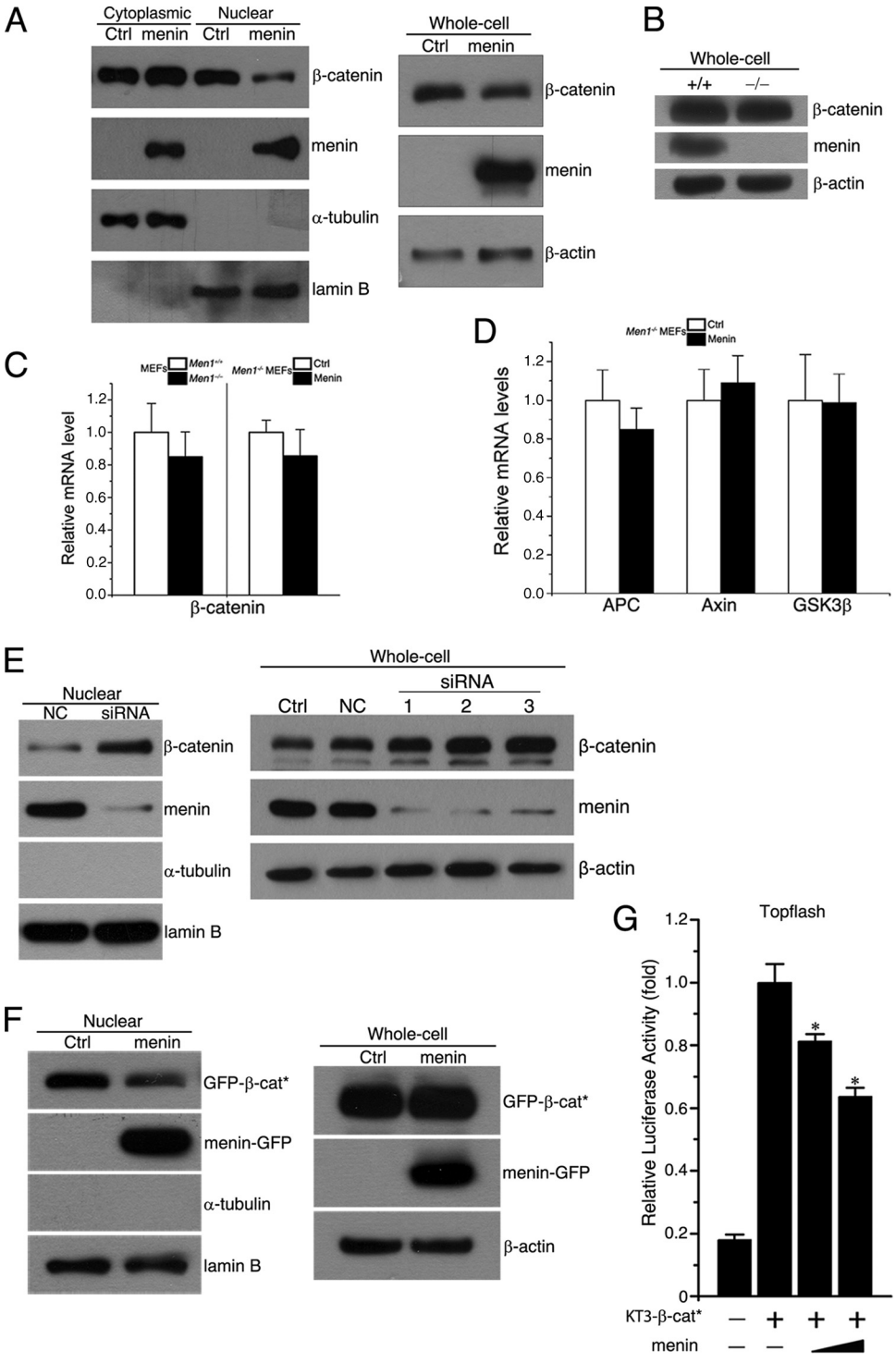


FIG. 4. Menin regulates subcellular distribution and activity of β-catenin. (A) *Men1*$^{-/-}$ MEFs were transfected with wild-type menin or vehicle vector. α-Tubulin and lamin B were used as loading controls for cytoplasmic and nuclear lysates, respectively. Endogenous nuclear β-catenin is reduced in menin-overexpressing *Men1*$^{-/-}$ MEF cells, and the total β-catenin is slightly reduced. (B) Endogenous β-catenin protein levels do not differ significantly between *Men1*$^{+/+}$ and *Men1*$^{-/-}$ MEFs. (C) RT-PCR quantification of β-catenin mRNA levels in *Men1*$^{+/+}$ MEFs, *Men1*$^{-/-}$ MEFs, and *Men1*$^{-/-}$ MEFs transfected with menin or vector control. (D) RT-PCR quantification of APC, axin, and GSK3β mRNA levels in *Men1*$^{-/-}$ MEFs transfected with menin or vector control. The results from three independent experiments are expressed as mean *n*-fold change ± SD. (E) Knockdown of menin by *Men1*-specific siRNA increases nuclear β-catenin accumulation in HeLa cells. Nonspecific siRNA (NC) was used as a control. Three pairs of *Men1* siRNA were tested. (F) 293T cells were cotransfected with menin-GFP, and stable-form GFP–β-catenin (GFP-β-cat*). pEGFP-N2 vehicle vector was used as a control. The nuclear β-catenin was dramatically reduced, whereas the total β-catenin was not changed. (G) Menin suppresses β-catenin/LEF-mediated transcriptional activation. 293T cells were transfected with TOP-flash or FOP-flash luciferase reporter plasmids, pRL-TK, KT3-β-cat*, and menin. The results obtained from three independent experiments were normalized and expressed as mean change ± SD. Cell lysates were all prepared at 48 h after transfection for immunoblotting assay.

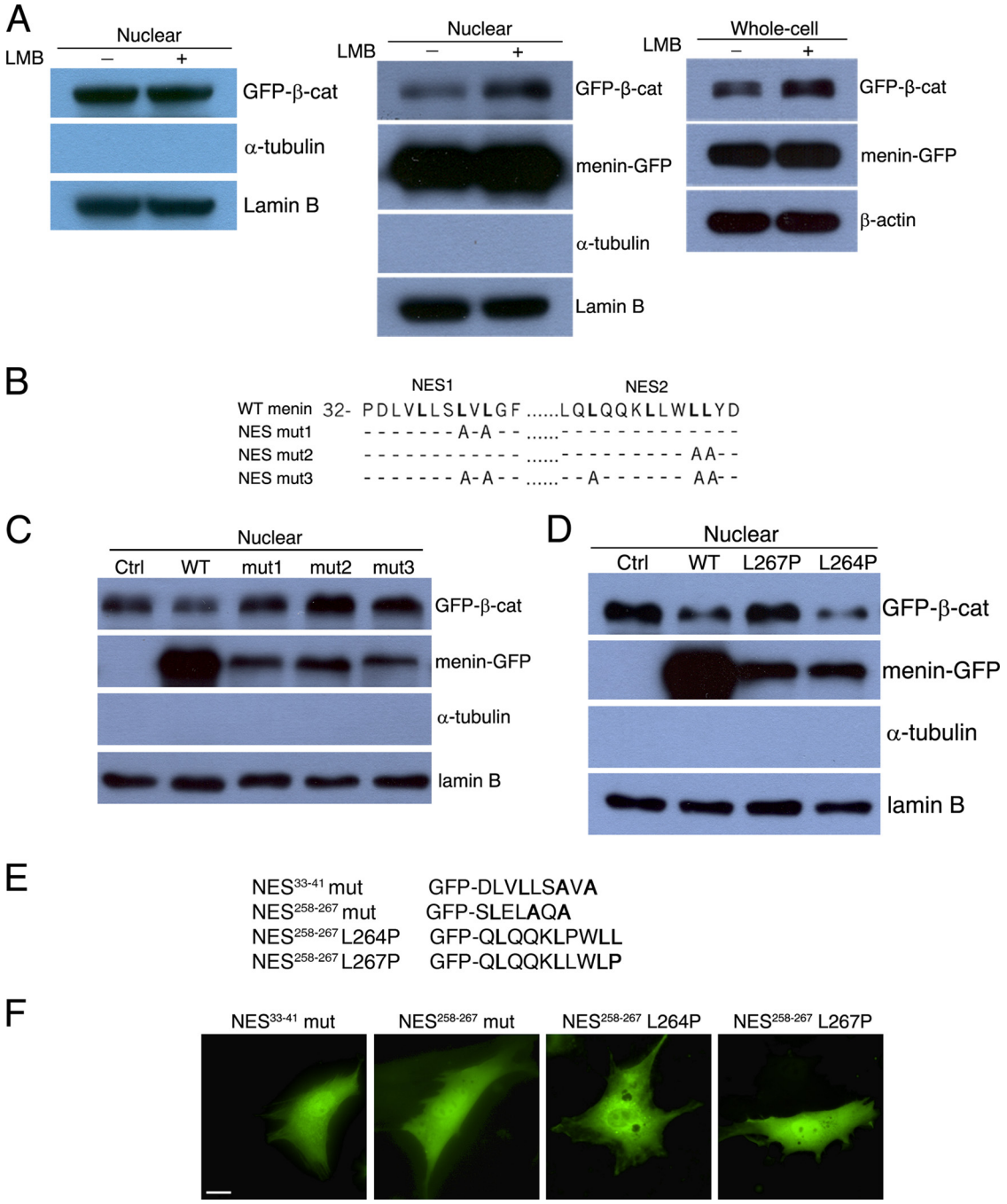


FIG. 5. Nuclear export functions of menin with NES mutations are impaired. (A) 293T cells were cotransfected with GFP–β-catenin and pEGFP-N2 control or menin-GFP and then treated with LMB (50 ng/ml) or mock treated for 24 h. Cells were harvested after 12 h treatment for immunoblotting. LMB treatment did not significantly affect nuclear GFP–β-catenin protein levels, whereas it blocked menin-mediated nuclear export and degradation of GFP–β-catenin. (B) Site-directed NES mutations were introduced in NES1 (NES mut1), NES2 (NES mut2), or both (NES mut3). (C) NES-mutated menin was co-overexpressed with GFP–β-catenin in 293T cells. Nuclear lysates were prepared at 24 h after transfection for immunoblotting. (D) Two menin-GFP mutants (L264P and L267P) derived from MEN1 patients were coexpressed with GFP–β-catenin in 293T cells. Nuclear lysates were prepared at 24 h after transfection for immunoblotting. (E) Mutations NES mut1, NES mut2, L264P, and L267P were introduced in GFP-NES fusions. (F) GFP-NES mutants were transfected into *Men1*$^{-/-}$ MEFs. The GFP fluorescence was observed at 24 h after transfection. The L264P mutant remained nuclear export function. Scale bar, 30 μm.

tant GFP-NES constructs were generated (Fig. 5E) and transfected in *Men1*$^{-/-}$ MEF cells. The mutant GFP-NES distribution was observed under a fluorescence microscope. This clearly showed that all GFP-NES mutants were localized in the nucleus and cytoplasm except the L264P mutant, which was located mainly in the cytoplasm (Fig. 5F). Altogether, these data indicated that NES masking of menin impaired its nuclear export function and led to β-catenin nuclear accumulation.

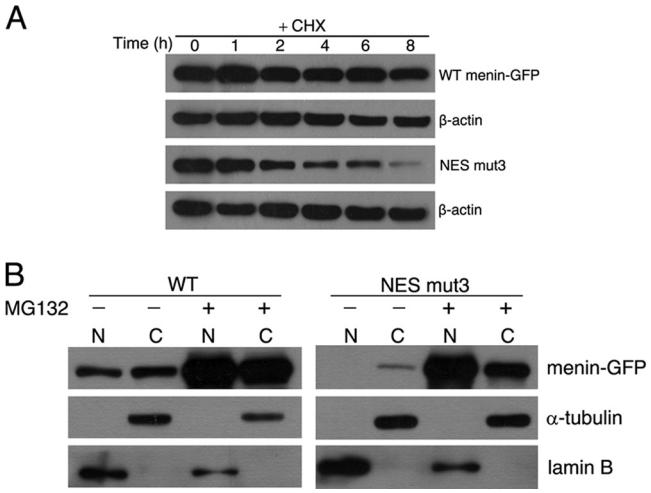


FIG. 6. Menin with NES mutations is rapidly degraded. (A) 293T cells expressing NES mut3 were treated with CHX (20 μg/ml), an inhibitor of de novo protein synthesis. Whole-cell lysates were prepared at the time points indicated for immunoblotting. NES mut3 displayed degradation in a time course pattern. (B) *Men1*$^{-/-}$ MEFs expressing wild-type (WT) menin or NES mut3 were treated with the proteasome inhibitor MG132 (25 μM) or mock treated at 24 h after transfection. Cytoplasmic and nuclear lysates were prepared after 6 h of treatment for Western blots.

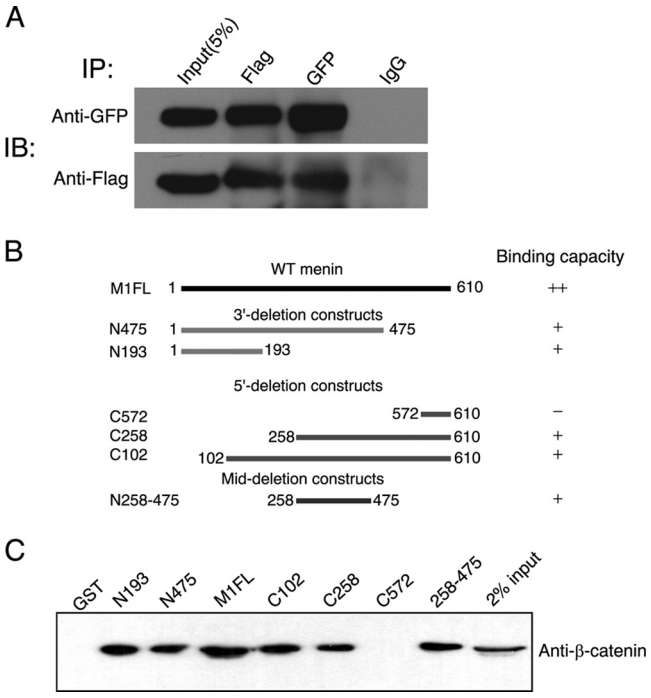


FIG. 7. The interaction between β-catenin and menin. (A) Coimmunoprecipitation of menin and β-catenin, which were overexpressed in 293T cells. Whole-cell lysates were prepared and immunoprecipitated with anti-Flag, anti-GFP, or control immunoglobulin G, respectively. (B) The full-length and truncated GST-menin constructs for mapping the binding of menin and β-catenin. The capacity of each menin fragment for binding to β-catenin is shown on the right. (C) Mapping of β-catenin binding sites in menin. GST pulldown assays were performed and analyzed with anti-β-catenin antibody.

It was noticed that menin mutants were unexpectedly degraded rapidly, which was confirmed by CHX chase experiments (Fig. 6A). NES mut3 was degraded in both the cytoplasm and nuclei of *Men1*$^{-/-}$ MEFs. The degradation could be reversed by MG132, a proteasome inhibitor (Fig. 6B). This is consistent with a previous study which demonstrated that menin mutants were rapidly degraded via the ubiquitin-proteasome pathway (43).

Menin interacts with β-catenin in vivo and in vitro. In order to investigate how menin drives β-catenin out of the nucleus, we examined the interaction of these two proteins through in vivo and in vitro binding assays. Immunoprecipitation assays showed that menin could be pulled down by β-catenin or vice versa (Fig. 7A). GST pulldown assays using purified GST-tagged full-length menin (GST-M1FL) and β-catenin were performed to examine their interaction in vitro. Several truncated GST-menin constructs were generated to map the binding sites for β-catenin (Fig. 7B), including 3′ deletions (N475 and N193), 5′ deletions (C572, C258, and C102), and a construct containing the middle part of menin (N258 to 475). Two binding sites were identified, in the N terminus (aa 1 to 193) and the middle (aa 258 to 475) (Fig. 7C). We also observed an intact interaction of patient-derived C-terminally truncated menin (GST-Arg415ter) with β-catenin (data not shown). Consistent with this, it was reported that the C-terminal activation domain and its adjacent ARM repeats of β-catenin could interact with menin (38).

DISCUSSION

A number of studies using different systems have demonstrated that the tumor suppressor menin is a crucial regulator of cell proliferation. Loss of menin leads to tumorigenesis in more than 10 different tissues in MEN1 syndrome, such as insulinoma, parathyroid, and pituitary adenoma, whereas overexpression of menin deteriorates pancreatic β-cell proliferation in gestational diabetes (20). It has been reported that menin upregulates $p18^{Ink4c}$ and $p27^{kip1}$ expression, dependent on histone H3K4 methylation (28, 37, 45). Menin also binds Smad3 protein and participates in transforming growth factor β-induced growth inhibition (18). Recent studies show that lack of menin leads to loss of E-cadherin on β-cell membranes, which indicates the activation of β-catenin signaling (4, 44) However, a study reported that suppression of *Men1* by siRNA reduces activity of Wnt signaling in vitro (6). The precise role of menin in Wnt/β-catenin signaling remains to be identified. In the present study, we revealed that β-catenin accumulated dramatically in the nuclei in *Men1*-deficient β cells in vivo and in *Men1*$^{-/-}$ MEFs as well, which were different from cells containing wild-type menin. Overexpression of menin markedly reduced β-catenin accumulation in the nuclei. Our results imply that Wnt/β-catenin signaling might be activated during β-cell proliferation in association with loss of menin.

It has recently been recognized that Wnt/β-catenin signaling plays an important role in pancreas development and β-cell proliferation. It has been reported that activation of Wnt signaling inhibits islet proliferation (6). However, downregulation of Wnt/β-catenin signaling by overexpression of axin also led to β-cell mass reduction (36). In mice stably expressing β-catenin

(β-*cat*^{active}), early expression of β-catenin disrupts pancreatic development, whereas late expression leads to an increase of islets (13). Knockout of β-catenin specifically in the pancreas resulted in a reduction of β-cell mass (9). Consistent with this, overexpression of GSK3β in vivo promotes β-catenin degradation and impairs β-cell proliferation. In an intestinal cell line, a reduction of menin increases the expression of cyclin D1, which is also a target gene of Wnt/β-catenin signaling (40). Our study has revealed that menin inhibits transcriptional activation of Wnt signaling. We speculate that menin may control cell proliferation through suppression of Wnt signaling in MEN1 patients.

The subcellular localization and activity of β-catenin are directly controlled by numerous molecules through regulating its stability and transport (8, 14, 35). Previous studies demonstrated that several tumor suppressors, such as APC and axin, shuttle between the nucleus and cytoplasm. The appropriate localization and shuttling are crucial for their functions in regulation of Wnt/β-catenin signaling. Menin is known as a nuclear protein containing three NLS (12, 23). We analyzed the menin sequence and identified three potential NES. Using GFP-NES fusion proteins, we identified two functional NES in two leucine zipper-like motifs. Heterokaryon formation assays and FRAP analysis demonstrated that wild-type menin could shuttle between the nucleus and cytoplasm in a CRM1-dependent way. It is notable that NES2 contains a complete LXXLL domain (aa 263 to 267), which was also responsible for the interaction of menin and other nuclear receptors (10). We propose that the two leucine zipper-like motifs should be crucial for menin functions, although the crystal structure of menin protein was not determined.

Moreover, our results showed that overexpression of menin could reduce exogenous stable β-catenin in the nucleus. The expression of APC, axin, and GSK3β was not significantly changed by menin. These data indicated that menin did not directly regulate β-catenin degradation. We speculate that menin could interact with β-catenin and promote β-catenin translocation from nucleus to cytoplasm. Nuclear β-catenin, which was exported by menin, could be degraded by the destruction complex in the cytoplasm or form an interaction on the plasma membrane (7). We further revealed that LMB not only blocked menin-mediated β-catenin nuclear export but also reduced β-catenin degradation, which indicated that menin-mediated β-catenin degradation was dependent on its nuclear export function. The nuclear export function of menin was impaired by NES mutations but was maintained in menin with intact NES. Nevertheless, mutated menin underwent a rapid degradation in a proteasome-dependent way, as reported previously (43). This is consistent with the observation that in MEN1 tumors *Men1* mutations lead to complete loss of menin protein and its functions.

In conclusion, menin regulates subcellular localization of β-catenin via nuclear-cytoplasmic shuttling. Loss of menin leads to Wnt/β-catenin signaling activation. Our results will help to better understand the development of MEN1 related tumors and to explore novel therapeutic approaches for insulinoma and even diabetes. However, the regulation and function of Wnt/β-catenin signaling in development and replication of pancreatic β cells need be further studied.

ACKNOWLEDGMENTS

This research is supported by grants from the Shanghai Education Commission (Y0204), Shanghai Committee of Science and Technology (08DZ2271000), Natural Science Foundation of China (08410706700, 30725037, and 30890043), and 973 project (2006CB503904).

We declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

REFERENCES

1. **Agarwal, S. K., S. C. Guru, C. Heppner, M. R. Erdos, R. M. Collins, S. Y. Park, S. Saggar, S. C. Chandrasekharappa, F. S. Collins, A. M. Spiegel, S. J. Marx, and A. L. Burns.** 1999. Menin interacts with the AP1 transcription factor JunD and represses JunD-activated transcription. Cell **96:**143–152.
2. **Barth, A. I., D. B. Stewart, and W. J. Nelson.** 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. USA **96:**4947–4952.
3. **Bertolino, P., I. Radovanovic, H. Casse, A. Aguzzi, Z. Q. Wang, and C. X. Zhang.** 2003. Genetic ablation of the tumor suppressor menin causes lethality at mid-gestation with defects in multiple organs. Mech. Dev. **120:**549–560.
4. **Bertolino, P., W. M. Tong, P. L. Herrera, H. Casse, C. X. Zhang, and Z. Q. Wang.** 2003. Pancreatic β-cell-specific ablation of the multiple endocrine neoplasia type 1 (MEN1) gene causes full penetrance of insulinoma development in mice. Cancer Res. **63:**4836–4841.
5. **Chandrasekharappa, S. C., S. C. Guru, P. Manickam, S. E. Olufemi, F. S. Collins, M. R. Emmert-Buck, L. V. Debelenko, Z. Zhuang, I. A. Lubensky, L. A. Liotta, J. S. Crabtree, Y. Wang, B. A. Roe, J. Weisemann, M. S. Boguski, S. K. Agarwal, M. B. Kester, Y. S. Kim, C. Heppner, Q. Dong, A. M. Spiegel, A. L. Burns, and S. J. Marx.** 1997. Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science **18:**404–407.
6. **Chen, G., M. Wang, S. Farley, L. Y. Lee, L. C. Lee, and M. P. Sawicki.** 2008. Menin promotes the Wnt signaling pathway in pancreatic endocrine cells. Mol. Cancer Res. **6:**1894–1907.
7. **Clevers, H.** 2006. Wnt/β-catenin signaling in development and disease. Cell **127:**469–480.
8. **Cong, F., and V. Varmus.** 2004. Nuclear-cytoplasmic shuttling of Axin regulates subcellular localization of β-catenin. Proc. Natl. Acad. Sci. USA **101:**2882–2887.
9. **Dessimoz, J., C. Bonnard, J. Huelsken, and A. Grapin-Botton.** 2005. Pancreas-specific deletion of β-catenin reveals Wnt-dependent and Wnt-independent functions during development. Curr. Biol. **15:**1677–1683.
10. **Dreijerink, K. M., K. W. Mulder, G. S. Winkler, J. W. Höppener, C. J. Lips, and H. T. Timmers.** 2006. Menin links estrogen receptor activation to histone H3K4 trimethylation. Cancer Res. **66:**4929–4935.
11. **Fabbro, M., and B. R. Henderson.** 2003. Regulation of tumor suppressors by nuclear-cytoplasmic shuttling. Exp. Cell Res. **282:**59–69.
12. **Guru, S. C., P. K. Goldsmith, A. L. Burns, S. J. Marx, A. M. Spiegel, F. S. Collins, and S. C. Chandrasekharappa.** 1998. Menin, the product of the MEN1 gene, is a nuclear protein. Proc. Natl. Acad. Sci. USA **95:**1630–1634.
13. **Heiser, P. W., J. Lau, M. M. Taketo, P. L. Herrera, and M. Hebrok.** 2006. Stabilization of β-catenin impacts pancreas growth. Development **133:**2023–2032.
14. **Henderson, B. R.** 2000. Nuclear-cytoplasmic shuttling of APC regulates β-catenin subcellular localization and turnover. Nat. Cell Biol. **2:**653–660.
15. **Heppner, C., K. Y. Bilimoria, S. K. Agarwal, M. Kester, L. J. Whitty, S. C. Guru, S. C. Chandrasekharappa, F. S. Collins, A. M. Spiegel, S. J. Marx, and A. L. Burns.** 2001. The tumor suppressor protein menin interacts with NF-κB proteins and inhibits NF-κB-mediated transactivation. Oncogene **20:**4917–4925.
16. **Hussein, N., J. Lu, H. Casse, S. Fontanière, A. M. Morera, S. M. Guittot, A. Calender, N. Di Clemente, and C. X. Zhang.** 2008. Deregulation of anti-Mullerian hormone/BMP and transforming growth factor-beta pathways in Leydig cell lesions developed in male heterozygous multiple endocrine neoplasia type 1 mutant mice. Endocrinol. Relat. Cancer **15:**217–227.
17. **Jin, T.** 2008. The WNT signalling pathway and diabetes mellitus. Diabetologia **51:**1771–1780.
18. **Kaji, H., L. Canaff, J. J. Lebrun, D. Goltzman, and G. N. Hendy.** 2001. Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type β signaling. Proc. Natl. Acad. Sci. USA **98:**3837–3842.
19. **Karnik, S. K., C. M. Hughes, X. Gu, O. Rozenblatt-Rosen, G. W. McLean, Y. Xiong, M. Meyerson, and S. K. Kim.** 2005. Menin regulates pancreatic islet growth by promoting histone methylation and expression of genes encoding p27^{Kip1} and p18^{INK4c}. Proc. Natl. Acad. Sci. USA **102:**14659–14664.
20. **Karnik, S. K., H. Chen, G. W. McLean, J. J. Heit, X. Gu, A. Y. Zhang, M. Fontaine, M. H. Yen, and S. K. Kim.** 2007. Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. Science **318:**806–809.
21. **Korinek, V., N. Barker, K. Willert, M. Molenaar, J. Roose, G. Wagenaar, M. Markman, W. Lamers, O. Destree, and H. Clevers.** 1998. Two members of

the Tcf family implicated in Wnt/beta-catenin signaling during embryogenesis in the mouse. Mol. Cell. Biol. **18:**1248–1256.
22. **Köster, M., T. Frahm, and H. Hauser.** 2005. Nucleocytoplasmic shuttling revealed by FRAP and FLIP technologies. Curr. Opin. Biotechnol. **16:**28–34.
23. **La, P., A. Desmond, Z. Hou, A. C. Silva, R. W. Schnepp, and X. Hua.** 2006. Tumor suppressor menin: the essential role of nuclear localization signal domains in coordinating gene expression. Oncogene **25:**3537–3546.
24. **Larsson, C., B. Skogseid, K. Oberg, Y. Nakamura, and M. Nordenskjöld.** 1988. Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature **332:**85–87.
25. **Liu, Z., K. Tanabe, E. Bernal-Mizrachi, and M. A. Permutt.** 2008. Mice with beta cell overexpression of GSK-3β have reduced beta cell mass and proliferation. Diabetologia **51:**623–631.
26. **Logan, C. Y., and R. Nusse.** 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. **20:**781–810.
27. **Major, M. B., 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, and R. T. Moon.** 2007. Wilms tumor suppressor WTX negatively regulates WNT/β-catenin signaling. Science **316:**1043–1046.
28. **Milne, T. A., C. M. Hughes, R. Lloyd, Z. Yang, O. Rozenblatt-Rosen, Y. Dou, R. W. Schnepp, C. Krankel, V. A. Livolsi, D. Gibbs, X. Hua, R. G. Roeder, M. Meyerson, and J. L. Hess.** 2005. Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. Proc. Natl. Acad. Sci. USA **102:**749–754.
29. **Morin, P. J., A. B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein, and K. W. Kinzler.** 1997. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science **275:**1787–1790.
30. **Murtaugh, L. C.** 2008. The what, where, when and how of Wnt/β-catenin signaling in pancreas development. Organogenesis **4:**81–86.
31. **Mussmann, R., M. Geese, F. Harder, S. Kegel, U. Andag, A. Lomow, U. Burk, D. Onichtchouk, C. Dohrmann, and M. Austen.** 2007. Inhibition of GSK3 promotes replication and survival of pancreatic beta cells. J. Biol. Chem. **282:**12030–12037.
32. **Persad, S., A. A. Troussard, T. R. McPhee, D. J. Mulholland, and S. Dedhar.** 2001. Tumor suppressor PTEN inhibits nuclear accumulation of beta-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation. J. Cell Biol. **153:**1161–1174.
33. **Piñol-Roma, S., and G. Dreyfuss.** 1993. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature **355:**730–732.
34. **Rodriguez, J. A., S. Schüchner, W. W. Au, M. Fabbro, and B. R. Henderson.** 2004. Nuclear-cytoplasmic shuttling of BARD1 contributes to its proapoptotic activity and is regulated by dimerization with BRCA1. Oncogene **23:** 1809–1820.
35. **Rosin-Arbesfeld, R., F. Townsley, and M. Bienz.** 2000. The APC tumour suppressor has a nuclear export function. Nature **406:**1009–1012.
36. **Rulifson, I. C., S. K. Karnik, P. W. Heiser, D. ten Berge, H. Chen, X. Gu, M. M. Taketo, R. Nusse, M. Hebrok, and S. K. Kim.** 2007. Wnt signaling regulates pancreatic β cell proliferation. Proc. Natl. Acad. Sci. USA **104:** 6247–6252.
37. **Schnepp, R. W., Y. X. Chen, H. Wang, T. Cash, A. Silva, J. A. Diehl, E. Brown, and X. Hua.** 2006. Mutation of tumor suppressor gene *Men1* acutely enhances proliferation of pancreatic islet cells. Cancer Res. **66:**5707–5715.
38. **Sierra, J., T. Yoshida, C. A. Joazeiro, and K. A. Jones.** 2006. The APC tumor suppressor counteracts β-catenin activation and H3K4 methylation at Wnt target genes. Genes Dev. **20:**586–600.
39. **Stommel, J. M., N. D. Marchenko, G. S. Jimenez, U. M. Moll, T. J. Hope, and G. M. Wahl.** 1999. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. EMBO J. **18:**1660–1672.
40. **Theillaumas, A., M. Blanc, C. Couderc, G. Poncet, W. Bazzi, C. Bernard, M. Cordier-Bussat, J. Y. Scoazec, and C. Roche.** 2008. Relation between menin expression and NF-kappaB activity in an intestinal cell line. Mol. Cell Endocrinol. **291:**109–115.
41. **Thyssen, G., T. H. Li, L. Lehmann, M. Zhuo, M. Sharma, and Z. Sun.** 2006. LZTS2 is a novel β-catenin-interacting protein and regulates the nuclear export of β-catenin. Mol. Cell. Biol. **26:**8857–8867.
42. **Welters, H. J., and R. N. Kulkarni.** 2008. Wnt signaling: relevance to β-cell biology and diabetes. Trends Endocrinol. Metab. **19:**349–355.
43. **Yaguchi, H., N. Ohkura, M. Takahashi, Y. Nagamura, I. Kitabayashi, and T. Tsukada.** 2004. Menin missense mutants associated with multiple endocrine neoplasia type 1 are rapidly degraded via the ubiquitin-proteasome pathway. Mol. Cell. Biol. **24:**6569–6580.
44. **Yan, J., Y. Yang, H. Zhang, C. King, H. M. Kan, Y. Cai, C. X. Yuan, G. S. Bloom, and X. Hua.** 2009. Menin interacts with IQGAP1 to enhance intercellular adhesion of beta-cells. Oncogene **28:**973–982.
45. **Yokoyama, A., T. C. Somervaille, K. S. Smith, O. Rozenblatt-Rosen, M. Meyerson, and M. L. Cleary.** 2005. The Menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. Cell **123:**207–218.
46. **Yu, X., and R. C. Malenka.** 2003. Beta-catenin is critical for dendritic morphogenesis. Nat. Neurosci. **6:**1169–1177.