CHAPTER 4

Cellular Functions of Menin

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Abstract

Since its discovery as a novel protein some 10 years ago, many cellular functions of menin have been identified. However, which ones of these relate specifically to menin's role as a tumor suppressor and which ones not remains unclear. Menin is predominantly nuclear and acts as a scaffold protein to regulate gene transcription by coordinating chromatin remodeling. It is implicated in both histone deacetylase and histone methyltransferase activity and, via the latter, regulates the expression of cell cycle kinase inhibitor and homeobox domain genes. TGF-β family members are key cytostatic molecules and menin is a facilitator of the transcriptional activity of their signaling molecules, the Smads, thereby ensuring appropriate control of cell proliferation and differentiation.

Introduction

The basic cell functions of menin will be reviewed. The focus will be on the role of menin in cell cycle regulation, DNA repair and chromatin remodeling. Whereas the primary structure of menin has been well conserved throughout evolution and orthologues are present in fruit fly, zebrafish and mouse, a menin homologue is apparently not present in nematodes and yeasts.

Cell Cycle

In the cell cycle, a gap (G1) phase is incorporated between nuclear division (M phase) and DNA synthesis (S phase); G2 phase occurs between S and M. Differentiated cells may exit G1 and enter a resting phase, G0. To enter S phase, activation of cyclin-dependent kinases (CDKs) is required. CDKs bind to a cyclin subunit to become catalytically competent and the cyclin-CDK complexes are tightly regulated. During G1 diverse signals are evaluated and on this basis the cell either enters S phase or enters G0 or undergoes apoptosis. The G2 phase is devoted to mending replication errors and ensuring that all is in order to proceed with mitosis. Oncogenic transformation is largely the result of malfunctions in these G1 and G2 mechanisms.

Before G1 and in the absence of mitogenic signals, CDK2 is kept inactive. In resting cells, E2F factors are bound to the retinoblastoma protein (Rb) or family members and inactivate them. Mitogens work by increasing D-type cyclins, which combine with CDK4 and CDK6 to phosphorylate and inactivate Rb. The E2Fs that are released activate transcription of genes encoding components supporting DNA replication.

Premature entry into S phase is prevented by inhibitors of the cyclin-CDK complexes. These cyclin dependent kinase inhibitors (CDKIs) include p15Ink4b, p16Ink4a, p18Ink4c, p21Cip1/WAF1, p27Kip1 and p57Kip2 and some may mediate cytostatic signals. On the other hand, mitogens can suppress the expression or location or activity of CDKIs. Mitogenic factors acting through receptor tyrosine kinases activate the Ras pathway to stimulate cell proliferation, growth

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SuperMEN1: Pituitary, Parathyroid and Pancreas, edited by Katalin Balogh and Attila Patocs.
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and survival. In the GTP-bound state, the Ras-MEK-ERK cascade promotes CDK activation. ERK phosphorylates and stabilizes the transcription factor, c-Myc, that induces and inhibits expression of cyclin D1 and CDKIs, respectively.

The cytokine TGF-β and family members provide cytostatic signals that limit G1 progression and cell proliferation. TGF-β activates a membrane complex of serine/threonine kinase receptors that phosphorylates Smad2 and Smad3 that associate with Smad4 and the complex translocates to the nucleus where it regulates transcription in combination with coactivators and corepressors. A subset of the regulated genes is critical for arresting G1. In epithelial cells this involves induction of CDKIs and repression of c-Myc. Smad-2, -3 and -4 are considered as tumor suppressors and mutations in several components of the TGF-β signaling pathway are contributors to a wide variety of cancers.

Menin and the Cell Cycle

Menin is a nuclear protein in nondividing (interphase) cells[1,2] and it is only in mitosis when the nuclear membrane has dissolved that menin appears in the cytoplasm.[3] At this time it may be associated with cytoskeletal elements. Menin interacts with nonmuscle myosin II-A heavy chain (NMHC II-A) that mediates alterations occurring in cytokinesis and cell shape during cell division[4] and also interacts with the intermediate filament network proteins, glial fibrillary acidic protein (GFAP) and vimentin.[5] It is unclear whether this relocalization represents only a sequestering of menin before cytokinesis or that the protein is playing a functional role in this location during late mitosis.

Tumor suppressors BRCA1 and BRCA2 are poorly expressed in quiescent cells. By contrast, we found that menin protein is relatively well expressed in quiescent rat pituitary somatolactotrope GH4C1 cells at G0-G1.[2] The CDKIs, such as p21 and p27, are also well expressed in quiescent cells and we suggested that menin may function like these CDKIs (or regulate their expression). We found that the levels of menin transiently decrease as Rb protein becomes hyperphosphorylated as cells enter the cell cycle and then increase again as the cells enter S phase from the G1-S-phase boundary onward (see Fig. 1). Others have identified increases in menin mRNA at this time.[6] It is at this stage that expression of other tumor suppressors such as BRCA1, BRCA2 and p53 increases. Thus menin may play some role at the G1-S-phase checkpoint analogous to BRCA1, BRCA2 and p53. It is to be emphasized, however, that the relative changes in menin expression are modest (2-3-fold) relative to the marked changes in expression noted for other tumor suppressors over the course of the cell cycle. Changes in post-translational modification that might suggest alterations in activity throughout the cell cycle have yet to be examined.

Menin and the Retinoblastoma Protein

Studies with knockout mice have provided evidence that menin and Rb may operate in a common pathway to regulate cell proliferation.[7] Mice homozygous for either deletion of the Rb1 gene or the *Men1* gene die in utero. Mice heterozygous for either deletion of the Rb1 gene[8] or the Men1 gene develop endocrine tumors.[9,10] In the $Rb1^{+/-}$ mice, intermediate pituitary and thyroid tumors occur frequently with less frequent development of pancreatic islet hyperplasia and parathyroid lesions. In the $Men1^{+/-}$ mice, pancreatic islet and anterior pituitary adenomas are common. In mice heterozygous for both Men1 and Rb1 deletion, pancreatic hyperplasia and tumors of the intermediate pituitary and thyroid occur at high frequency. The tumor spectrum in the double heterozygotes is a combination of those for the individual heterozygotes, with no decrease in age of onset. This suggests that menin and Rb function in a common pathway. This would be in contrast with studies of mice heterozygous for deletion of Rb1 and p53 that exhibit accelerated tumorigenesis and a broadening of the spectrum of tumor types observed to encompass all the types exhibited in the individual heterozygotes.[11]

Menin and CDK Inhibitors

p18-p27 double mutant mice, like Rb heterozygous mice, develop multiple endocrine neoplasias, including pituitary, thyroid, parathyroid and adrenal, providing evidence that p18 and p27

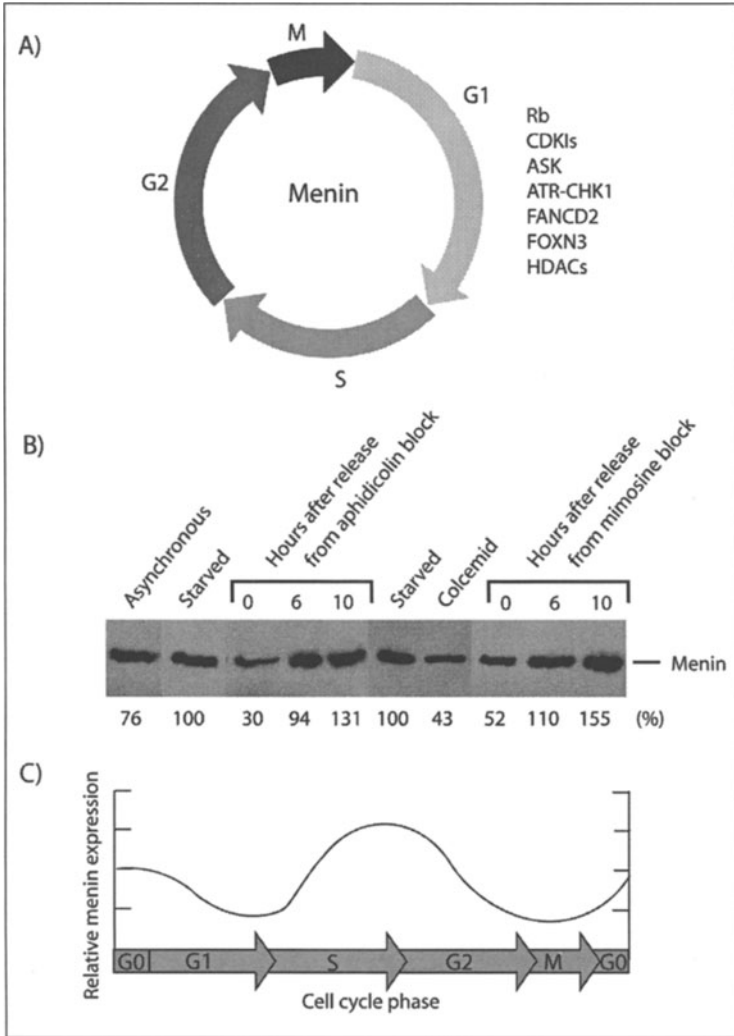

Figure 1. Menin affects the activity and/or expression of several key cell cycle regulators either by directly interacting with them or modulating transcription of their genes. A) G0/G1 to S phase transition; Menin blocks the transition from G0/G1 to S phase and appears to function in a common pathway with the retinoblastoma (Rb) protein. Menin is required for the expression of CDKIs such as p18 and p27 that maintain cells in a quiescent state. S phase: Menin inhibits cell proliferation by interacting with activator of S-phase kinase (ASK). Cell cycle checkpoints (G1 to S; and G2 to M) and DNA repair: Menin can bind DNA directly (like BRCA1) and functions in DNA repair via the ATR-CHK1, FANCD2 or FOXN3 (CHES1) pathways. For some of these, histone deacetylase (HDAC) complexes are also involved. For further details see text. B) Menin protein expression changes throughout the cell cycle. Rat somatolactotroph GH4C1 cells were serum starved for 24 h, cultured in complete media containing either aphidicolin or mimosine (G1-S block), or colcemid (G2-M block) for 24 h and then released from blockade by culture in complete media for the indicated times (h). For experimental details see reference 2. Relative expression levels (%) of menin protein were determined by SDS-PAGE and immunoblot of cell extracts. C) Relative expression of menin protein throughout the cell cycle with peak levels at the intra-S phase.

function by regulating Rb's tumor suppressor function. The types of endocrine tumors cover the spectrum seen in MEN1 and MEN2.[12,13] However, mice lacking p53 develop lymphomas and soft tissue sarcomas.[11] p53 functions as a checkpoint gene to monitor genomic integrity, whereas Rb functions to integrate mitogenic signals to determine whether the cell will enter S phase or not.

Menin directly regulates the expression of the CDKIs, p27 and p18. Menin does this by recruiting mixed lineage leukemia (MLL) protein complexes to their gene promoters and coding regions. Loss of function of either MLL or menin results in down-regulation of p27 and p18 expression and deregulated cell proliferation.[14] By use of a mouse model with heterozygous deletion of the *Men1* gene, it was shown that menin-dependent histone methylation maintained expression of CDKIs and prevented the formation of pancreatic islet tumors.[15] Therefore, menin is involved in cell proliferation control by an epigenetic mechanism. Excision of Men1 in mouse embryonic fibroblasts (MEFs) accelerated entry into S phase accompanied by increased CDK2 activity and decreased expression of p18 and p27. Complementation of menin-null cells with menin repressed S-phase entry.[16]

In stable Men1-deficient Leydig tumor cell lines reconstituted menin expression decreased cell proliferation with a block in transition from G0/G1 to S phase and an increase in apoptosis accompanied by increased p18 and p27 expression.[17] Tissue-specific inactivation of Men1 in neural crest precursor cells in the mouse leads to perinatal death with cleft palate and other cranial bone defects associated with decreased p27 expression.[18] The study demonstrated that menin functions in vivo during osteogenesis and is required for palatogenesis, skeletal rib formation and perinatal viability.

Therefore, a recurring theme arising from studies investigating the functions of menin either early in embryogenesis and fetal development, on the one hand and those examining roles that when lost in the growing or adult organism lead to endocrine (and other) neoplasia, on the other, has been the requirement of menin for the proper functioning of some of the CDKIs in cell cycle control.

Menin and GTPases

Ras-transformed murine fibroblasts (NIH3T3 cells) demonstrate increased proliferation, clonal formation in soft agar and tumor growth after inoculation into nude mice. Overexpression of menin in these cells caused them to partially revert to the phenotype of the parent NIH3T3 cells in vitro and in vivo.[19] The studies support menin acting as a tumor suppressor, although the activities being displayed by menin in these experiments may not necessarily involve a direct antagonism of the Ras pathway.

It has been suggested that nucleoside diphosphate (NDP) kinases might act as molecular switches to alter cell fate towards proliferation or differentiation in response to external signals.[20] The activity of the NDP kinases would be regulated by small molecular weight G proteins like Rad and Rac that function as guanosine triphosphate (GTP)ases. While menin is not of small molecular weight it has been shown to interact with the tumor suppressor NM23H1/NDP kinase. While neither protein has GTPase activity of its own, their interaction induces GTPase activity by menin.[21,22] It has been proposed that menin has several motifs similar to those in known GTPases although the homology is weak. It would be anticipated that the interaction with menin stimulates GEF activity of nm23 although this remains unknown. In addition NDP kinases function at the plasma membrane and evidence is lacking for menin being in the appropriate cellular localization to fulfil this function.

Menin and JunD

JunD is a member of the activator protein-1 (AP-1) transcription factor family and in contrast to other Jun and Fos proteins has antimitogenic activity. JunD negatively regulates fibroblast proliferation and antagonizes transformation by Ras in vitro and in vivo.[23] Of all the AP-1 family members, menin interacts only with JunD and represses its transcriptional activity by association with an mSin3A-histone deacetylase (HDAC) complex.[24-27] We had pointed out that it appeared paradoxical that one antimitogenic factor would reduce the activity of another.[2] We suggested

that whereas menin is a regulator of JunD action, JunD may not be the main mediator of menin action. Further studies in fibroblasts have suggested that the nature of JunD can change depending upon whether it is bound by menin when it functions as a growth suppressor or it is not bound by menin in which case it acts as a growth promoter like other AP-1 family members.[28] However, the result of JunD-menin interaction may be cell-type specific as JunD has a differentiating effect in osteoblasts, an action that is inhibited by menin.[29]

Menin and Activator of S-Phase Kinase (ASK)

Targeted disruption of the *Men1* gene leads to enhanced cell proliferation, whereas complementation of menin-null cells with menin reduces cell proliferation. Menin interacts with activator of S-phase kinase (ASK), a component of the Cdc7/ASK kinase complex. The C-terminal domain of menin interacts with ASK. Wild-type menin completely represses ASK-induced cell proliferation although it does not affect the steady-state cell cycle profile of ASK-infected cells.[30] As menin itself represses basal cell proliferation, it is unclear whether menin's effects are occurring via its interaction with ASK or in an unrelated manner. Also, ASK itself did not alter the steady-state cell cycle profile. Disease-related C-terminal menin mutants that do not interact with ASK did not repress either ASK-induced or basal cell proliferation. From other studies it appears that ASK is in the nucleus.[31] The C-terminal menin deletion mutants would not gain access to the nucleus. Further studies need to be done to determine where in the cell any physical interaction between menin and ASK is taking place and what the exact functional link between the two proteins is.

Menin and TGF-β Family Members

In most mature tissues the cytokine TGF-β provides cytostatic signals that limit G1 progression and cell proliferation. Menin is a Smad3-interacting protein and is a facilitator of transcriptional activity of the Smads (see Fig. 2).[32] In anterior pituitary cells, inactivation of menin blocks TGF-β and activin signaling, antagonizing their proliferation-inhibitory properties.[33,34] In cultured parathyroid cells from uremic hemodialysis patients in which the menin signaling pathways are intact, menin inactivation achieved by menin antisense oligonucleotides leads to loss of TGF-β inhibition of parathyroid cell proliferation and parathyroid hormone (PTH) secretion (see Fig. 3). Moreover, TGF-β does not affect the proliferation and PTH production of parathyroid cells from MEN1 patients that were devoid of menin protein.[35,36] Antisense inhibition of menin in a rat duodenal crypt-like cell line increased cell proliferation with loss of cell-cycle arrest in G1 and increased expression of cyclin D1 and CDK4 and decreased expression of the TGF-β Type II receptor.[37] Hence, menin plays a critical role in mediating the cytostatic effects of TGF-β ligands.

During early embryogenesis and fetal development and in some adult mesenchymal cells, TGF-β and bone morphogenetic proteins (BMPs) play important roles. Homozygous Men1 inactivation in mice is embryonic lethal and the fetuses exhibit cranial and facial developmental defects. Cranial bones form by intramembranous ossification and menin may play an important role in this type of bone formation. In vitro, menin promotes the initial commitment of multipotential mesenchymal stem cells to the osteoblast lineage through interactions with the BMP-2 signaling molecules, Smad1/Smad5 and the key osteoblast regulator, Runx2, whereas the interaction of menin and the TGF-β signaling molecule, Smad3, inhibits later osteoblast differentiation by negatively regulating the BMP-Runx2 cascade.[38,39] The focus in these studies was predominantly on bone differentiation markers. However, it would also be important to extend these studies by evaluating menin's influence on cell cycle markers. In vivo, in the mouse, tissue-specific inactivation of Men1 in neural crest cells that contribute to cranial bones and the skeletal ribs and other tissues leads to defects in osteogenesis and perinatal death.[18] The fact that mice and humans heterozygous for loss of the *Men1* gene develop normally indicates haplosufficiency for all of menin's normal functions.[9,10] However, for some functions and in some cell types only, further reduction in menin results in developmental deficits.

The deregulation of the TGF-β family pathway has also been correlated with Men1 inactivation and altered cell growth in vivo by studying the Leydig cell tumors of heterozygous Men1 mutant mice.[40] In the cells of the tumors the anti-Mullerian hormone (AMH)/BMP pathway was

impaired with reduced expression of AMH receptor Type 2, decreased expression of Smad1, -3, -4 and -5 and reduced BMP transcriptional activity. The expression of p18 and p27 was reduced and that of CDK4 increased. In other studies, it was noted that Men1-null MEFs demonstrate reduced expression of extracellular matrix proteins critical for organogenesis and that are induced by TGF-β.[41] TGF-β failed to stimulate expression of these proteins in the menin-null MEFs that also had poor responsiveness to TGF-β induced Smad3-mediated transcription.

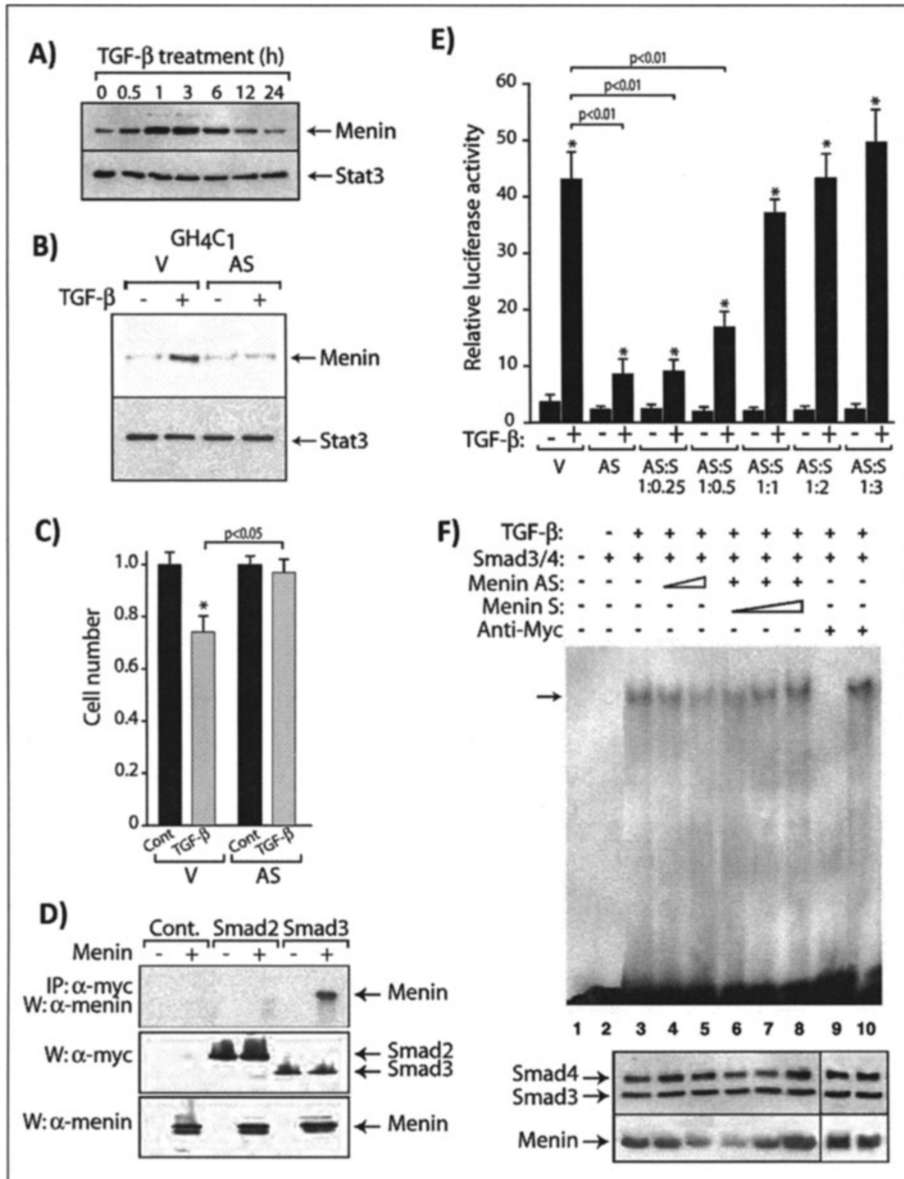

Figure 2. Legend viewed on following page.

Figure 2, viewed on previous page. Role of menin in TGF-β-mediated cell proliferation and gene transcription facilitated by Smad/DNA interaction. A) TGF-β stimulates menin expression. Serum-starved GH4C1 cells were cultured in TGF-β for the indicated times (h) and total cell menin levels were measured by immunoblot after SDS-PAGE with Stat3 as the protein loading control. B) Endogenous menin expression is suppressed by antisense menin cDNA. Serum-starved GH4C1 cells stably transfected with either vector alone (V) or antisense menin (AS) were cultured in the absence (-) or presence (+) of TGF-β for 1h. Menin expression was assessed by immunoblotting of cell extracts after SDS-PAGE. C) Antisense menin blocks the TGF-β-induced inhibition of pituitary cell proliferation. Serum-starved GH4C1 cells were cultured without (Cont.) or with TGF-β for 72 h and cell numbers were counted. D) Menin specifically binds the TGF-β signaling molecule, Smad3. Menin was transfected into COS7 cells with the indicated myc-tagged Smad2 or Smad3 constructs. Cell extracts were immunprecipitated with anti-myc antibodies followed by SDS-PAGE and immunoblotting with anti-menin antibodies. Total cell expression of the Smads and menin was monitored. W, Western blot; IP, immunoprecipitation. E) Antisense menin inhibits TGF-β-mediated transcriptional responses. The TGF-β-responsive promoter-luciferase reporter construct, 3TP-Lux, was transfected into HepG2 cells together with empty vector (V) or antisense menin (AS) either alone or with sense menin (S) and the cells were stimulated (+) or not (-) with TGF-β. Relative luciferase activity was measured and the mean values are shown. F) Reduced menin expression disrupts Smad3 binding to DNA. GH4C1 cells were transfected with myc-Smad3 and flag-Smad4 in the absence (-) or presence (+) of antisense menin alone or with sense menin. Nuclear extracts were subjected to electromobility shift assay. The shifted band (arrow), the Smad/DNA complex, was decreased in intensity by antisense menin and restored by coexpression of sense menin and was completed abolished by anti-myc antibodies. Lane 10 represents an extract from cells transfected with untagged (rather than myc-tagged) Smad3. Menin and Smad3/4 in the nuclear extracts were monitored by immunoblot. For experimental details see reference 33.

Cell Cycle Checkpoints and DNA Repair

Tumor suppressors like BRCA1, BRCA2 and p53 play key roles in protection against genomic instability. They integrate with components of DNA damage checkpoints such as ataxia telangiectasia mutated kinase (ATM) and ATM and Rad3-related kinase (ATR) whose substrates mediate cell cycle arrest, DNA repair or cell death. Menin may also share some of these functions.

Menin and Genomic Instability

A role for menin in the maintenance of genomic stability is suggested. Chromosomal instability (increased chromosomal breakage) was observed in cultured lymphocytes and in fibroblasts derived from skin biopsies of MEN1 patients and hence heterozygous for an MEN1 mutation.[42] Peripheral lymphocytes from MEN1 patients displayed an increase relative to normal controls in premature centromere division after exposure to the alkylating agent diepoxybutane (DEB) that crosslinks DNA.[43-45] Menin-deficient MEFs were also moderately sensitive to DEB and displayed a high frequency of chromosomal aberrations after exposure to this agent.[46] Studies in Drosophila showed that flies mutant for the Men1 orthologue are hypersensitive to ionization radiation and are hypermutable.[46] A genome-wide loss of heterozygosity (LOH) screening of 23 pancreatic lesions from 13 MEN1 patients has shown multiple allelic deletions indicating that MEN1 pancreatic tumors fail to maintain DNA integrity and demonstrate signs of chromosomal instability.[47] However, in another study, no obvious chromosomal instability was observed in islet cells of Men1 knockout mice and tumors developed in the absence of chromosome or microsatellite instability.[48]

Menin, DNA Binding and ATR-CHK1 Pathway

Menin binds DNA and interacts with proteins implicated in DNA damage pathways. The canonical cellular response to UV-induced damage involves activation of the ATR kinase pathway. Following UV irradiation of human embryonic kidney (HEK293) cells, menin concentration in chromatin increased but was decreased by the ATR inhibitor, caffeine.[49] Transfection of constitutively active checkpoint kinase 1 (CHK1) increased chromatin-bound menin mimicking the effect of UV irradiation and implicating the involvement of an ATR-CHK1 dependent pathway.

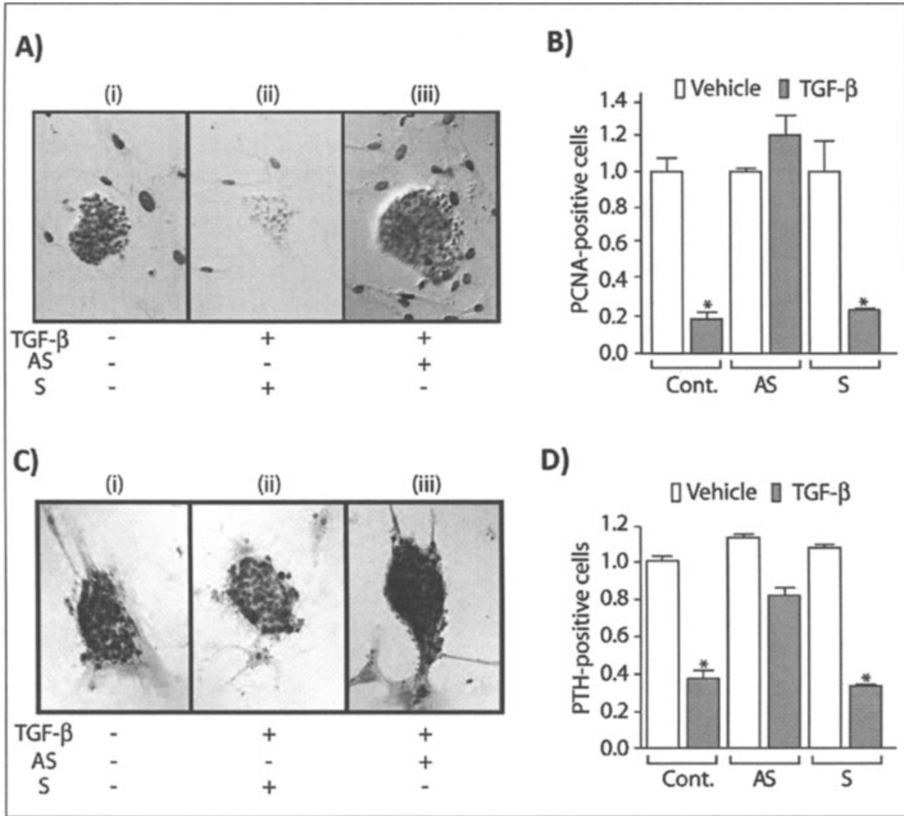

Figure 3. Role of menin in TGF-β-mediated inhibition of parathyroid cell proliferation and parathyroid hormone (PTH) production. Parathyroid cells (from patients with secondary hyperparathyroidism in which menin function is normal) were cultured in chamber slides without (–) or with (+) antisense (AS) or sense (S) menin oligos for 6 h. A) Cells were then cultured in fresh media without (–) or with (+) antisense or sense oligos without (–) or with (+) TGF-β for an additional 24 h and proliferating cell nuclear antigen (PCNA) immunocytochemistry performed. Representative views of cells cultured (i) without TGF-β or oligos, (ii) with TGF-β and sense oligos or (iii) with TGF-β and antisense oligos. B) Mean values for PCNA-positive cells for each group described in (A) without (vehicle) or with TGF-β. C) After the initial culture of cells for 6 h (described above) PTH immunocytochemistry was performed on some cells. Representative views of cells cultured as described under (B). D) Mean values of PTH-positive cells for each group described under (C) cultured without (vehicle) or with TGF-β. For experimental details see reference 35.

Similar to other tumor suppressors like BRCA1,[50] that are involved in DNA repair pathways, menin apparently directly binds dsDNA in a sequence independent way.[51] Amino acid sequences located towards the C-terminus of menin within the nuclear localization signals (NLS1 and NLS2) appear to be essential for this binding. The study was conducted in MEFs null for Men1 in which a failure to repress cell proliferation and cell cycle progression at the G2/M phase was noted. An anticipated effect at G1/S as occurs with BRCA1 depletion was not observed. However, the authors noted that the method of immortalization of the MEFs would have blocked the Rb pathway so that effects on this part of the cell cycle would not have been evaluated.

Menin and FANCD2

The FANCD2 protein is involved in DNA repair and mutations in it result in the inherited cancer syndrome, Fanconi's Anemia. Menin interacts with FANCD2; gamma-irradiation enhances the interaction and increases the accumulation of menin in the nuclear matrix.[52] These results suggest a role for menin, in cooperation with FANCD2, in DNA repair.

More recently, Marek et al[53] have compared the mutation frequency and spectra of Men1 and FANCD2 mutants in Drosophila. Men1 mutant flies were extremely prone to single base deletions within a homopolymeric tract, whereas FANCD2 mutants displayed large deletions. Neither overexpression nor loss of Men1 modified the interstrand crosslink (ICL) sensitivity of FANCD2 mutants. The different mutation spectra of Men1 and FANCD2 mutants together with lack of evidence for genetic interaction between these genes indicates that Men1 plays an essential role in ICL repair distinct from the Fanconi anemia genes.

Menin and RPA2

Menin directly binds to and colocalizes in the nucleus with the 32-kDa subunit (RPA2) of replication protein A (RPA), a heterotrimeric protein required for DNA replication, recombination and repair.[54] The interactive region was mapped to the N-terminal portion of menin and menin bound preferentially in vitro to free RPA2 rather than the RPA heterotrimer. Menin had no effect on RPA-DNA binding in vitro. However, menin antibodies coimmunoprecipitated RPA1 with RPA2 from HeLa extracts suggesting that menin binds to the RPA heterotrimer or a novel RPA1-RPA2-containing complex in vivo. The functional consequences of the interactions are unclear but it is possible that menin acts as a scaffold protein to bridge different components of a cross-link repair network. Menin could also have, by its association with HDACs, a more general effect on chromatin remodeling facilitating access of damaged DNA to the repair machinery.

Menin and FOXN3 (CHES1)

FOXN3 (CHES1), a member of the forkhead/winged-helix transcription factor family, was originally identified by its ability to suppress DNA damage sensitivity phenotypes in checkpoint-deficient yeast strains. By study of integrity of DNA damage checkpoints in mutant Drosophila lacking the Men1 orthologue and in MEFs deficient for Men1, biochemical and genetic interactions between menin and FOXN3 (CHES1) were demonstrated.[55] FOXN3 (CHES1) is part of a transcriptional repressor complex, that includes mSin3a, HDAC1 and HDAC2 and it interacts with menin in a DNA damage-responsive S-phase checkpoint pathway.

Chromatin Remodeling

Transcription factors bind to DNA in a sequence-specific manner and they recruit cofactors like chromatin-modifying complexes to regulate transcription of specific genes. In the nucleus, DNA is wrapped around histone proteins to form nucleosomes and the repeating nucleosomes form the chromatin fibres of chromosomes. Accessibility to chromosomal DNA is a prerequisite for gene transcription by RNA polymerases. Post-translational modifications of the tails of histones involving acetylation and methylation influence the status of nucleosomes and affect the recruitment of transcriptional cofactor complexes.

Menin and Transcriptional Regulation

Menin is implicated in the regulation of many genes via interaction with specific transcription factors and with the large subunit of RNA polymerase II.[56] Menin exerts a dual role, either as a repressor or as an activator depending upon the particular transcription factor involved. This might be explained by a model in which menin serves to link transcription regulation with chromatin modification.

Menin and Histone Deacetylase

The chromatin modification, histone acetylation, is correlated with activation of gene transcription. Histone deacetylation mediated by complexes of the general transcription repressor, Sin3A,

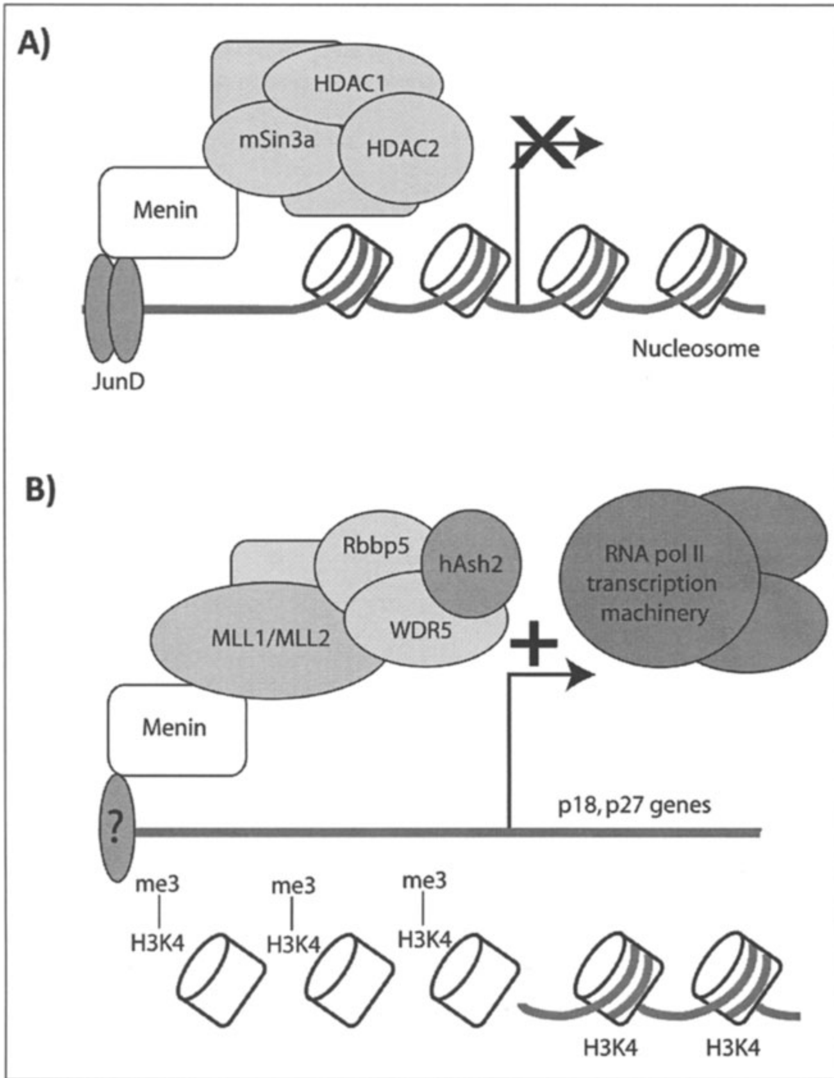

Figure 4. Menin regulates gene transcription. A) Menin interacts with JunD and represses JunD-mediated transcription by recruiting a histone-deacetylating complex comprising the mSin3A, HDAC1 and HDAC2 proteins. B) Menin is a component of MLL1 or MLL2 complexes with histone methyltransferase (H3K4) activity. Other proteins in the complex are WDR5, Rbbp5 and hASH2. Recruitment by an unknown DNA binding factor of menin and the histone methyl transferase complex leads to histone 3 lysine 4 trimethylation (H3K4-me3) coincident with the presence of RNA polymerase II and the basal transcription machinery at promoters such as those for the CDKI, p18 and p27, genes.

with HDACs is related to gene inactivation. Menin interacts with HDAC-mSin3A complexes to repress transcription of JunD (see Fig. 4A).[25,26]

Menin and Histone Methyltransferase

Menin, as a component of the MLL chromatin remodeling complexes, is involved in trimethylation of the fourth lysine (K) residue of histone H3 (H3K4 trimethylation) that is strongly associated with transcription activation.[56,57] By recruiting histone methyltransferase activity menin acts as a tumor suppressor by activating transcription of antiproliferative genes like those for CDKIs (see Fig. 4B).[14,15]

Menin binds to the N-terminal region of MLL1. Some but not all of the few MEN1 associated missense mutants tested fail to bind MLL.[56] So it remains unclear exactly how essential the loss of H3K4 methylation is in MEN1 tumorigenesis. Of interest, fusion proteins created by chromosomal translocations of MLL and other proteins in the complex have a causal role in several forms of leukemia. Menin is an essential cofactor for MLL-associated leukemogenesis.[58-60] This has prompted the suggestion that menin, usually considered as a tumor suppressor, under special circumstances may promote oncogenesis.

Homeobox-domain (HOX) genes that control the fundamental body plan during embryogenesis are targets for MLL. Consistent with menin being part of the MLL complexes, the protein is located at the promoters of some HOX genes and shown in some cases to regulate individual HOX gene expression in various cells including MEFs,[56] mouse bone marrow cells[59] and HeLa cells.[57,58,61] Deregulation of expression of many and a few HOX genes has been found in parathyroid tumors of patients with MEN1 and sporadic hyperparathyroidism, respectively.[62] The mechanisms underlying the altered HOX gene expression and whether it is causal of or coincidental to the tumorigenesis remains to be established.

Menin, Chromatin and Gene Expression

By combining chromatin immunoprecipitation (ChIP) assay with gene expression analysis, hundreds of menin-occupied chromatin regions were revealed.[63] The majority (67%) were at known genes (promoters, internal, 3' regions) with 33% located outside known gene regions. While this reinforces the notion of menin as a transcriptional regulator, menin likely acts in an indirect fashion (it may not bind DNA directly) by functioning as a scaffold protein within chromatin remodeling complexes. Knowledge of the participation of menin in regulating several genes has come from ChIP analysis and promoter-reporter transfection assays. Additional information has come from microarray analysis of a variety of Men1 expressing versus Men1 deleted cells. Interestingly, this has revealed that menin target genes in one tissue show minimal overlap with targets of other tissues.[63]

Genomic binding sites of menin and other proteins representing MLL complexes (see above) were mapped to several thousand gene promoters in three different cell types by ChIP coupled with microarray analyses.[61] While menin frequently colocalized with chromatin modifying complexes it also bound other promoters by other (unknown) mechanisms.

Conclusion

It is becoming clear that menin plays critical roles in embryogenesis and early fetal development for which functions menin appears to be haplosufficient. Menin is involved in organogenesis of neural tube, heart and craniofacial structures and hematopoiesis. In the adult, reductions in menin expression, under the influence of the hormone prolactin, has been implicated in the normal expansion of pancreatic islet β-cells that occurs in pregnancy to meet the increased insulin demand at this time.[64]

Acknowledgements

Work from our laboratories has been supported by the Canadian Institutes of Health Research (CIHR) (Grant MOP-9315 to G.N.H.) and the Kanzawa Medical Research Foundation (to H.K.) and the Ministry of Science, Education and Culture of Japan (Grant-in-aid 15590977 to H.K.).

References

1. Guru SC, Goldsmith PK, Burns AL et al. Menin, the product of the MEN1 gene, is a nuclear protein. Proc Natl Acad Sci USA 1998; 95:1630-4.
2. Kaji H, Canaff L, Goltzman D et al. Cell cycle regulation of menin expression. Cancer Res 1999; 59:5097-101.
3. Huang SC, Zhuang Z, Weil RJ et al. Nuclear/cytoplasmic localization of the multiple endocrine neoplasia type 1 gene product, menin. Lab Invest 1999; 79:301-10.
4. Obungu VH, Burns AL, Agarwal SK et al. Menin, a tumor suppressor, associates with nonmuscle myosin II-A heavy chain. Oncogene 2003; 22:6347-58.
5. Lopez-Egido J, Cunningham J, Berg M et al. Menin's interactions with glial fibrillary acidic protein and vimentin suggests a role for the intermediate filament network in regulating menin activity. Exp Cell Res 2002; 278:175-83.
6. Ikeo Y, Sakurai A, Suziki R et al. Proliferation-associated expression of the MEN1 gene as revealed by in situ hybridization: possible role of the menin as a negative regulator of cell proliferation under DNA damage. Lab Investig 2000; 80:797-804.
7. Loffler KA, Biondi CA, Gartside G et al. Lack of augmentation of tumor spectrum or severity in dual heterozygous Men1 and Rb1 knockout mice. Oncogene 2007; 26:4009-17.
8. Nitikin AY, Juarez-Perez MI, Li S et al. RB-mediated suppression of spontaneous multiple neuroendocrine neoplasia and lung metastases in Rb+/– mice. Proc Natl Acad Sci USA 1999; 96:3916-21.
9. Crabtree JS, Scacheri PC, Ward JM et al. A mouse model of multiple endocrine neoplasia type 1, develops multiple endocrine tumors. Proc Nat Acad Sci USA 2001; 98:1118-23.
10. Bertolino P, Tong WM, Galendo D et al. Heterozygous men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1. Mol Endocrinol 2003; 17:1880-92.
11. Harvey M, Vogel H, Lee EYH et al. Mice deficient in both p53 and Rb develop tumors primarily of endocrine origin. Cancer Res 1995; 55:1146-51.
12. Franklin DS, Godfrey VL, Lee H et al. CDK inhibitors p18INK4c and p27 Kip1 mediate two separate pathways to collaboratively suppress tumorigenesis. Genes Develop 1998; 12:2899-911.
13. Franklin DS, Godfrey VL, O'Brien DA et al. Functional collaboration between different cyclin-dependent kinase inhibitors suppress tumor growth with distinct tissue specificity. Mol Cell Biol 2000; 20:6147-58.
14. Milne TA, Hughes CM, Lloyd R et al. Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. Proc Natl Acad Sci USA 2005; 102:749-54.
15. Karnik SK, Hughes CM, Gu X et al. Menin regulates pancreatic islet growth by promoting histone methylation and expression of genes encoding p27Kip1 and p18INK4c. Proc Natl Acad Sci USA 2005; 102:14659-64.
16. Schnepp RW, Chen YX, Wang H et al. Mutation of tumor suppressor men1 acutely enhances proliferation of pancreatic islet cells. Cancer Res 2006; 66:5707-15.
17. Hussein N, Casse H, Fontaniere S et al. Reconstituted expression of menin in men1-deficient mouse leydig tumour cells induces cell cycle arrest and apoptosis. Eur J Cancer 2007; 43:402-14.
18. Engleka KA, Wu M, Zhang M et al. Menin is required in cranial neural crest for palatogenesis and perinatal viability. Dev Biol 2007; 311:524-37.
19. Kim YS, Burns AL, Goldsmith PK et al. Stable overexpression of MEN1 suppresses tumorigenicity of RAS. Oncogene 1999; 18:5936-42.
20. Kimura N, Shimada N, Ishijima Y et al. Nucleoside diphosphate kinases in mammalian signal transduction systems: recent development and perspective. J Bioenerg Biomembr 2003; 35:41-7.
21. Ohkura N, Kuhi M, Tsukada T et al. Menin, a gene product responsible for multiple endocrine neoplasia type 1, interacts with the putative tumor metastasis suppressor nm23. Biochem Biophys Res Commun 2001; 282:1206-10.
22. Yaguchi H, Ohkura N, Tsukada T et al. Menin, the multiple endocrine neoplasia type 1, gene product, exhibits GTP-hydrolysing activity in the presence of the tumor metastasis suppressor nm23. J Biol Chem 2002; 277:38197-204.
23. Pfarr CM, Mechta F, Spyrou G et al. Mouse JunD negatively regulates fibroblast growth and antagonizes transformation by ras. Cell 1994; 76:747-60.
24. Agarwal SK, Guru SC, Heppner C et al. Menin interacts with the AP1 transcription factor JunD and represses JunD-activated transcription. Cell 1999; 96:143-52.
25. Gobl AE, Berg M, Lopez-Egido LR et al. Menin represses JunD-activated transcription by a histone deacetylase-dependent mechanism. Biochim Biophys Acta 1999; 1447:51-6.
26. Kim H, Lee JE, Cho EJ et al. Menin, a tumor suppressor, represses JunD-mediated transcriptional activity by association with an mSin3A-histone deacetylase complex. Cancer Res 2003; 63:6135-9.
27. Yazgan O, Pfarr CM. Differential binding of the menin tumor suppressor protein to JunD isoforms. Cancer Res 2001; 61:916-20.

28. Agarwal SK, Novotny EA, Crabtree JS et al. Transcriptional factor JunD, deprived of menin, switches from growth suppressor to growth promoter. Proc Natl Acad Sci USA 2003; 100:10770-5.
29. Naito J, Kaji H, Sowa H et al. Menin suppresses osteoblast differentiation by antagonizing the AP-1 factor, JunD. J Biol Chem 2005; 280:4785-91.
30. Schnepp RW, Hou Z, Wang H et al. Functional interaction between tumor suppressor menin and activator of S-phase kinase. Cancer Res 2004; 64:6791-6.
31. Sato N, Sato M, Nakayama M et al. Cell cycle regulation of chromatin binding and nuclear localization of human Cdc7-ASK kinase complex. Gene Cell 2003; 8:451-63.
32. Hendy GN, Kaji H, Sowa H et al. Menin and TGF-β superfamily member signaling via the Smad pathway in pituitary, parathyroid and osteoblast. Horm Metab Res 2005; 37:375-9.
33. Kaji H, Canaff L, Lebrun JJ et al. Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type-β signaling. Proc Natl Acad Sci USA 2001; 98:3837-42.
34. Lacerte A, Lee EH, Reynaud R et al. Activin inhibits pituitary prolactin expression and cell growth through Smads, pit-1 and menin. Mol Endocrinol 2004; 18:1558-1569.
35. Sowa H, Kaji H, Kitazawa R et al. Menin inactivation leads to loss of transforming growth factor-β inhibition of parathyroid cell proliferation and parathyroid hormone secretion. Cancer Res 2004; 64:2222-8.
36. Naito J, Kaji H, Sowa H et al. Expression and functional analysis of menin in a multiple endocrine neoplasia type 1 (MEN1) patient with somatic loss of heterozygosity in chromosome 11q13 and unidentified germline mutation of the MEN1 gene. Endocr 2006; 29:485-90.
37. Ratineau C, Bernard C, Poncet G et al. Reduction of menin expression enhances cell proliferation and is tumorigenic in intestinal epithelial cells. J Biol Chem 2004; 279:24477-84.
38. Sowa H, Kaji H, Canaff L et al. Inactivation of menin, the product of the multiple endocrine neoplasia type 1 gene, inhibits the commitment of multipotential mesenchymal stem cells into the osteoblast lineage. J Biol Chem 2003; 278:21058-69.
39. Sowa H, Kaji H, Hendy GN et al. Menin is required for bone morphogenetic protein 2- and transforming growth factor β-regulated osteoblastic differentiation through interaction with Smads and Runx2. J Biol Chem 2004; 279:40267-75.
40. Hussein N, Lu JL, Casse H et al. Deregulation of anti-Mullerian hormone/BMP and transforming growth factor-β pathways in leydig cell lesions developed in male heterozygous multiple endocrine neoplasia type 1 mutant mice. Endocrine-Related Cancer 2008; 15:217-27.
41. Ji Y, Prasad NB, Novotny EA et al. Mouse embryo fibroblasts lacking the tumor suppressor menin show altered expression of extracellular matrix protein genes. Mol Cancer Res 2007; 5:1041-51.
42. Scappaticci S, Maraschio P, del Ciotto N et al. Chromosome abnormalities in lymphocytes and fibroblasts of subjects with multiple endocrine neoplasia type 1. Cancer Genet Cytogenet 1991; 52:85-92.
43. Tomassetti P, Cometa G, Del Vecchio E et al. Chromosomal instability in multiple endocrine neoplasia type 1. Cytogenetic evaluation with DEB test. Cancer Genet Cytogenet 1995; 79:123-6.
44. Sakurai A, Katai M, Itakura Y et al. Premature centromere division in patients with multiple endocrine neoplasia type 1. Cancer Genet Cytogenet 1999; 109:138-40.
45. Itakura Y, Sakurai A, Katai M et al. Enhanced sensitivity to alkylating agent in lymphocytes from patients with multiple endocrine neoplasia type 1. Biomed Pharmacother 2000; 54(Suppl 1):187s-90s.
46. Busygina V, Suphapeetiporn K, Marek LR et al. Hypermutability in a drosophila model for multiple endocrine neoplasia type 1. Hum Mol Genet 2004; 13:2399-408.
47. Hessman O, Skogseid B, Westin G et al. Multiple allelic deletions and intratumoral genetic heterogeneity in MEN1 pancreatic tumors. J Clin Endocrinol Metab 2001; 86:1355-61.
48. Scacheri PC, Kennedy AL, Chin K et al. Pancreatic insulinomas in multiple endocrine neoplasia, type I knockout mice can develop in the absence of chromosome instability or microsatellite instability. Cancer Res 2004; 64:7039-44.
49. Farley SM, Chen G, Guo S et al. Menin localizes to chromatin through an ATR-CHK1 mediated pathway after UV-induced DNA damage. J Surg Res 2006; 133:29-37.
50. Paull TT, Cortez D, Bowers B et al. Direct DNA binding by brca1. Proc Natl Acad Sci USA 2001; 98:6086-91.
51. La P, Silva AC, Hou Z et al. Direct binding of DNA by tumor suppressor menin. J Biol Chem 2004; 279:49045-54.
52. Jin S, Mao H, Schnepp RW et al. Menin associates with FANCD2, a protein involved in repair of DNA damage. Cancer Res 2003; 63:4204-10.
53. Marek LR, Kottemann MC, Glazer PM et al. MEN1 and FANCD2 mediate distinct mechanisms of DNA crosslink repair. DNA Repair (Amst) 2008; 7:476-86.
54. Sukhodolets KE, Hickman AB, Agarwal SK et al. The 32-kilodalton subunit of replication protein A interacts with menin, the product of the MEN1 tumor suppressor gene. Mol Cell Biol 2003; 23:493-509.

55. Busygina V, Kottemann MC, Scott KL et al. Multiple endocrine neoplasia type 1 interacts with forkhead transcription factor CHES1 in DNA damage response. Cancer Res 2006; 66:8397-403.
56. Hughes CM, Rozenblatt-Rosen O, Milne TA et al. Menin associates with a trithorax family histone methyltransferase complex and with the hoxc8 locus. Mol Cell 2004; 13:587-97.
57. Yokoyama A, Wang Z, Wysocka J et al. Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate hox gene expression. Mol Cell Biol 2004; 24:5639-49.
58. Yokoyama A, Somervaille TC, Smith KS et al. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. Cell 2005; 123:207-18.
59. Chen YX, Yan J, Keeshan K et al. The tumor suppressor regulates hematopoiesis and myeloid transformation by influencing hox gene expression. Proc Natl Acad Sci USA 2006; 103:1018-23.
60. Caslini C, Yang Z, El-Osta M et al. Interaction of MLL amino terminal sequences with menin is required for transformation. Cancer Res 2007; 67:7275-83.
61. Scacheri PC, Davis S, Odom DT et al. Genome-wide analysis of menin binding provides insights into MEN1 tumorigenesis. PloS 2006; 2:e51.
62. Shen H-CJ, Rosen JE, Yang LM et al. Parathyroid tumor development involves deregulation of homeobox genes. Endocrine-Related Cancer 2008; 15:267-75.
63. Agarwal SK, Impey S, McWeeney S et al. Distribution of menin-occupied regions in chromatin specifies a broad role of menin in transcriptional regulation. Neoplasia 2007; 9:101-7.
64. Karnik SK, Chen H, McLean GW et al. Menin controls growth of pancreatic β-cells in pregnant mice and promotes gestational diabetes mellitus. Science 2007; 318:806-9.