Published in final edited form as:
Mol Cell Endocrinol. 2007 February ; 265-266: 34–41.

In Search of Tumor Suppressing Functions of Menin

Yuqing Yang and **Xianxin Hua***
Abramson Family Cancer Research Institute, Signal Transduction Program, Department of Cancer Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160, USA

Abstract

Human hereditary tumor syndromes serve as an ideal model for studying molecular pathways regulating tumorigenesis. Multiple endocrine neoplasia type 1 (MEN1), a human familial tumor syndrome, results from mutations in the *Men1* gene. *Men1* encodes a novel tumor suppressor, menin, of unknown biochemical function. Recently, significant progress has been made in identifying menin as a regulator of gene transcription, cell proliferation, apoptosis, and genome stability, leading to a new model of understanding menin's tumor-suppressing function. These findings suggest that menin's diverse functions depend on its association with chromatin and its control over gene transcription. This knowledge will likely be translated into new strategies to improve therapeutic interventions against MEN1 and other related cancers.

Keywords

menin; MEN1; cell proliferation; apoptosis; genome stability; transcription; epigenetics

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an inherited tumor syndrome characterized by development of tumors in multiple endocrine organs including the parathyroid glands, pancreatic islets, and the pituitary gland, and also in some non-endocrine organs (Marx et al., 1999b,Pannett and Thakker, 1999). MEN1 was first described as an autosomal dominant familial syndrome, and the gene mutated in MEN1 patients, *MEN1*, was identified in 1997 (Chandrasekharappa et al., 1997,Lemmens et al., 1997). *Men1* encodes a novel protein, menin, of unknown biochemical function. Because menin does not display an obvious homology to any known protein motifs, it is challenging to elucidate how menin functions as a tumor suppressor.

To date, over 300 germline mutations in MEN1 patients have been identified (Leotlela et al., 2003,Marx et al., 1999b,Pannett and Thakker, 1999). Tumors from MEN1 patients with one mutated germline *MEN1* allele often lose the normal *MEN1* allele (loss of heterozygosity, LOH). Mice heterozygous for the disrupted *Men1* allele also develop tumors in various endocrine glands with LOH of the *Men1* allele in the tumors, closely resembling the human MEN1 syndrome (Crabtree et al., 2001,Bertolino et al., 2003). These observations indicate that menin is a *bona fide* tumor suppressor.

*Corresponding author: Xianxin Hua, Abramson Family Cancer Research Institute, Signal Transduction Program, Department of Cancer Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160, USA. Phone 215-746-5565; Fax 215-746-5525; email huax@mail.med.upenn.edu

Emerging evidence suggests that menin plays a vital role in regulation of gene transcription, cell proliferation, apoptosis, and genome stability, which are among the hallmarks dysregulated in cancer cells. These observations provide novel insights into how menin suppresses tumorigenesis. In the present review, we will focus on the recent progress in understanding how menin regulates cellular homeostasis and functions as a tumor suppressor. In particular, how menin modulates gene transcription, cell proliferation, apoptosis and genome stability will be critically evaluated.

Regulation of gene transcription

Numerous studies demonstrate a crucial role for menin in regulating gene transcription. For instance, menin interacts with a number of transcriptional factors such as JunD, NF-κB, Smad3, and homeobox-containing DNA binding protein Pem (Poisson et al., 2003), and inhibits the activities of JunD and NF-κb (Agarwal et al., 1999,Heppner et al., 2001). Ectopic expression of menin inhibits promoter activity of the prolactin and insulin genes in pituitary tumor cells or insulinoma cells, respectively (Namihira et al., 2002,Sayo et al., 2002). Ectopic menin expression also inhibits insulin-induced endogenous *c-Fos* expression (Yumita et al., 2003). Several reports have recently shown that menin binds to the loci of several menin-dependent genes, including $p18^{ink4c}$, $p27^{kip1}$, *Hoxa9*, and *Hoxc8*, and regulates the transcription of those genes (Chen et al., 2006,Hughes et al., 2004,Karnik et al., 2005,Milne et al., 2005,Yokoyama et al., 2005). Genetic evidence also reinforces an essential role for menin in regulation of various endogenous genes. For example, ablation of *Men1* reduces the expression of $p27^{kip1}$, $p18^{ink4c}$, *caspase 8* and *Hoxc8* in mouse embryonic fibroblasts (MEF)(Hughes et al., 2004,Milne et al., 2005,Schnepp et al., 2004b), but enhances the expression of *insulin-like growth factor binding protein 2* (*IGFBP-2*) (La et al., 2004a), a gene involved in regulation of cell proliferation (Hoeflich et al., 2001). Complementing the menin-null cells with menin restores optimal expression of *caspase 8* and represses the *IGFBP-2* expression. Together, these studies strongly suggest an essential role for menin in regulating the transcription of endogenous genes.

Menin has been shown to associate with chromatin (Farley et al., 2006,Jin et al., 2003) and bind multiple endogenous genes including *hTERT* and *Hoxc8* (Hughes et al., 2004,Lin and Elledge, 2003). Menin also interacts with nuclear proteins such as transcription factors, histone methyltransferases (HMT) (Hughes et al., 2004,Kim et al., 2003) and histone deacetylases (HDAC). Thus, menin may function as a scaffold protein to regulate transcription of its target genes by associating with one of several of these various interacting proteins. These multiple interactions of menin and the various nuclear proteins may facilitate regulation of gene transcription and cellular homeostasis (Fig. 1).

Regulation of gene transcription by menin via associating with histone methyltransferases

Menin regulates gene transcription at least in part by modulating chromatin structure. Menin has been shown to associate with a protein complex containing Drosophila *trithorax*-like histone lysine methyltransferases, the mixed lineage leukemia (*MLL*) gene products, MLL and MLL2, both of which are SET domain-containing methyltransferases (Hughes et al., 2004,Yokoyama et al., 2004). This complex contains multiple proteins that are homologous to the members of the yeast SET1 complex (COMPASS) and three mammalian SET1-like complexes, including activating signal cointegrator 2 complex (ASCOM), the HCF-1 complex and the MLL complex (Hughes et al., 2004), which had previously been found to methylate histone H3 lysine 4 (H3K4) and activate gene transcription. The menin-interacting complex isolated from mouse embryonic fibroblasts (MEF) also methylates H3K4 *in vitro* (Hughes et al., 2004). These results support a model that menin recruits histone methyltransferases (HMT) and thus upregulates gene transcription (Fig.1A).

The menin complex has recently been shown to upregulate *p18ink4c* and *p27kip1* transcription by upregulating H3K4 methylation at the *p18ink4c* and *p27kip1* loci in both cultured cells and the murine pancreatic islets (Karnik et al., 2005,Milne et al., 2005). We and others have also showed that menin-HMT complex bind to the *Hoxa9* locus *in vivo* and promotes H3K4 methylation at the *Hoxa9* locus (Chen et al., 2006,Yokoyama et al., 2005). The trimethylated H3K4 recruits chd1, a methylated H3K4-specific binding protein of a chromatin remodeling complex, and activates gene transcription via chromatin remodeling (Chen et al., 2006,Pray-Grant et al., 2005).

The interaction between menin and HMT suggests that menin facilitates epigenetic regulation of gene transcription by histone modifications. It is likely that the cooperation between menin and MLL enhances the activity of menin in epigenetic control of gene expression (Fig. 1A). However, it is still unclear whether caspase 8, one of menin's target genes, is also regulated by H3K4 methylation. It is noteworthy that menin may regulate gene transcription in an MLL-independent manner (Scacheri et al., 2006), and how menin regulates gene transcription independent of MLL needs to be further investigated.

Regulation of gene transcription by menin via association with histone deacetylases

Acetylation of histones, which usually leads to activation of gene transcription, can be reversed by histone deacetylases that remove the acetyl group on histones, resulting in repression of the gene locus (Jenuwein and Allis, 2001). In contrast to the role of menin as an activator of *caspase 8* and *Hoxc8*, menin inhibits the activity of transcription factor JunD (Agarwal et al., 1999). Interestingly, this inhibition can be relieved by a histone deacetylase (HDAC) inhibitor (Gobl et al., 1999,Kim et al., 2003). This suggests that menin may inhibit the transcription of certain genes via recruiting histone deactylases to the promoter regions. Consistent with this notion, menin has been shown to associate with several HDACs and represses the expression of a reporter gene driven by a JunD binding site (Kim et al., 2003).

Menin represses expression of endogenous genes such as *hTERT* and *IGFBP-2* and associates with the JunD binding site in the *hTERT* promoter. Thus, it is likely that menin associates with JunD on the *hTERT* promoter, further recruits histone deacetylases to the promoter and downregulates the *hTERT* transcription (Fig. 1B) (Lin and Elledge, 2003,Kim et al., 2003). However, it is not yet clear whether HDACs inhibit menin's endogenous target genes in a menin-dependent manner. It is also possible that menin represses promoter regions through other histone modifications. For instance, methylation of lysine 9 and lysine 27 of histone H3 or acetylation of lysine 12 of histone H4 has been shown to change histone codes and repress gene transcription (Cao and Zhang, 2004,Jenuwein and Allis, 2001). Thus, detailed mechanisms underlying menin-mediated histone modifications and gene repression remain to be investigated.

Regulation of cell proliferation

Ectopic expression of menin inhibits proliferation of oncogenic Ras-transformed NIH3T3 cells, and suppresses tumorigenesis in nude mice transplanted with Ras-transformed cells (Kim et al., 1999). Overexpression of menin also represses proliferation of insulinoma cells and human endocrine tumor cells (Stalberg et al., 2004,Sayo et al., 2002). Moreover, immortalized *Men1*$^{-/-}$ MEFs display enhanced cell proliferation while complementing the *Men1*$^{-/-}$ cells with menin reduces cell proliferation, providing genetic evidence for menin's role in suppressing cell proliferation (La et al., 2004b,Schnepp et al., 2004a). In these studies, menin's effect on cell proliferation was measured by monitoring the accumulation of cell number after stable ectopic menin expression. It is still not clear whether transient or stable menin expression in these cells arrests or slows down cell cycle progression at a particular phase of the cell cycle.

Consistent with a role for menin in repressing cell proliferation, knock-down of menin expression increases cell proliferation in rat intestinal epithelial cells (Ratineau et al., 2004). Cell cycle profile analysis on these menin knockdown cells reveals that the number of cells in G1 phase reduces, while the number of cells in S-phase increases. In agreement with a role for menin in suppressing G1 to S phase transition, menin knockdown increases gene expression of *cyclin D1*, *cyclin D3* and cyclin D-dependent kinase 4 (CDK4) (Ratineau et al., 2004), which form a functional protein kinase that promotes cell cycle transition from G1 to S phase (Sherr, 1996) (Fig. 2). Further evidence supporting menin as a regulator of cell proliferation is that menin is also essential for JunD-mediated inhibition of cell proliferation (Agarwal et al., 2003). However, how menin affects the kinetics of G1-S phase transition, and whether restoration of menin in menin knockdown cells can block or decrease the G1-S phase transition remains unclear. To definitively determine that menin is vital for regulating G1 to S-phase transition, ideally it should be shown that ablation of *Men1* in cells accelerates G1 to S transition while complementing *Men1*$^{-/-}$ cells with wild type menin represses this G1 to S- transition.

Menin has been shown to functionally interact with activator of S-phase kinase (ASK), a mammalian homologue of yeast Dbf-4 that is an essential regulatory component of cdc7 protein kinase (Schnepp et al., 2004a). Cdc7/ASK is essential for DNA replication, most likely because it phosphorylates certain mini-chromosome maintenance (MCM) proteins (Masai et al., 2000). MEN1-derived mutations abolish not only menin's binding to ASK but also menin's repression on ASK- induced cell proliferation (Schnepp et al., 2004a). Thus, menin can repress cell proliferation by inhibiting ASK (Fig. 2). It is not yet exactly clear how menin regulates ASK. It is possible that menin affects the distribution of Cdc7/ASK to the origin of DNA replication. On the other hand, we and others have recently shown that menin plays a crucial role in upregulating expression of cyclin-dependent kinase inhibitors p18^{ink4c} and p27^{kip1}, and menin is recruited to the loci of these genes (Karnik et al., 2005,Milne et al., 2005,Schnepp et al., 2006). We further demonstrated that *Men1* abrogation increased cyclin-dependent kinase 2 (CDK2) activity and accelerated S phase entry in MEFs (Schnepp et al., 2006). The crucial role of menin in repressing S-phase entry has also been demonstrated in pancreatic islets *in vivo*, and increased S phase entry was observed as early as 7 days post *Men1* excision (Schnepp et al., 2006). Thus, menin has a crucial role in repressing G0/1 to S phase in pancreatic islet cells, perhaps through multiple mechanisms involving p18^{ink4c}, p27^{kip1}, CDK2 and ASK (Fig. 2).

Menin has also been shown to cooperate with transforming growth factor-β (TGF-β), a potent inhibitor of epithelial cells, and suppress cell proliferation of a rat pituitary cell line (Kaji et al., 2001). Menin interacts with Smad3 protein, which is activated by TGF-β and plays an important role in TGF-β-induced growth inhibition (Kaji et al., 2001). Consistent with these observations, reduced menin expression also leads to loss of TGF-β-mediated growth inhibition on primary parathyroid cells (Sowa et al., 2004). These results suggest that menin also inhibits cell proliferation by cooperating with other signaling pathways. However, it is unclear whether targeted disruption of *Men1* attenuates or blocks TGF-β-induced anti-proliferation. Adding more complexity to the menin-mediated repression on cell proliferation, JunD, a menin-interacting partner, has been shown to inhibit cell growth in a menin-dependent manner in MEFs (Agarwal et al., 2003). It remains unclear whether this function depends on regulating p27^{kip1}-CDK2 axis or not.

Thus, menin may regulate cell cycle progression in several distinct ways. Menin may regulate the p18^{ink4c}, p27^{kip1}/CDK2 pathway as well as the CDK4/cylin D pathway. In addition, it can also functionally repress ASK to regulate DNA replication as well as TGF-β-mediated suppression of cell proliferation (Fig. 2). However, whether all these pathways are crucial in regulating endocrine cells *in vivo* remains to be further investigated.

Regulation of apoptosis

Menin mediates apoptosis in MEFs (Schnepp et al., 2004b). Many tumor suppressors, including p53 and BRCA1, also regulate apoptosis. Infection of cells using menin-expressing adenoviruses, but not control viruses, triggers apoptosis (Schnepp et al., 2004b). Menin-induced apoptosis requires Bax and Bak, two proapoptotic proteins that form a gateway for multiple apoptotic pathways (Lindsten et al., 2000), suggesting that menin activates an apoptotic pathway that depends on Bax and Bak (Lindsten et al., 2000). Targeted deletion of *Men1* causes increased resistance to TNF-α-induced apoptosis, further supporting a vital role for menin in regulating apoptosis, (Schnepp et al., 2004b).

Interestingly, menin induces expression of caspase 8, an essential component in death receptor-related apoptotic pathways (Varfolomeev et al., 1998). Ablation of *Men1* in MEFs diminishes the *caspase 8* expression, while complementing menin-null cells with menin enhances the caspase 8 enzymatic activities in response to TNF-α treatment (Schnepp et al., 2004b). As a result, the menin-expressing cells have greater sensitivity to TNF-α mediated apoptosis, as compared to the menin-null cells (Schnepp et al., 2004b). Consistent with these findings, ectopic expression of menin in an insulinoma cell line also increases the number of the cells stained by Annexin V, a hallmark for apoptosis (Sayo et al., 2002). These results suggest that menin suppresses tumorigenesis by promoting apoptosis, at least in part, by modulating caspase 8 expression.

Caspase 8 is an essential initiator caspase that is activated by multiple death-receptors (Chen and Goeddel, 2002). Upon death ligand-mediated ligation of the death receptors, caspase 8 forms oligomers and then autocleaves itself into active form (Chen and Goeddel, 2002). The activated caspase 8 can cleave various effector caspases and initiate apoptosis, while targeted disruption of *caspase 8* in mouse leads to resistance to T cell apoptosis induced by death-receptors (Varfolomeev et al., 1998).

The promoter of human *caspase 8* is activated by transcription factors Sp1, ETS-like proteins, and tumor suppressor p53 (Liedtke et al., 2003). The *caspase 8* gene is frequently inactivated in neuroblastoma, in which caspase 8 is either deleted or silenced by DNA methylation (Teitz et al., 2000). Thus, these tumors are often resistant to death receptor-mediated apoptosis, and reintroduction of the caspase 8 cDNA into the tumor cells restores their sensitivity to death receptor-mediated apoptosis (Hopkins-Donaldson et al., 2000,Hopkins-Donaldson et al., 2003,Teitz et al., 2000). In addition, caspase 8 expression is also differentially inactivated by DNA methylation in its promoter region in many types of pediatric neuroblastomas, small cell lung cancer, their derived cell lines, and neuroendocrine lung cancers (Harada et al., 2002). Treating these cells with 5-aza-2 deoxycytidine, a nucleotide analog that inhibits DNA methylation, restores both t expression of caspase 8 in the cells and their sensitivity to caspase 8-dependent apoptosis (Fulda et al., 2001,Hopkins-Donaldson et al., 2003). Collectively, these studies suggest a vital role for caspase 8 in suppression of tumorigenesis.

Menin may potentiate apoptosis induced by various death receptor ligands, such as TNF-α, TRAIL, and Fas ligand, which promotes UV-induced skin cancer in mice (Hill et al., 1999). Menin may sensitize apoptosis by lowering the threshold for death receptor-mediated apoptosis and other apoptotic signals via maintaining optimal *caspase 8* expression, although it is not yet clear whether menin prevents the *caspase 8* promoter DNA from being methylated. Consistent with this notion, menin activates expression of the *caspase 8* gene and enhances the cell sensitivity to TNF-α-induced apoptosis, but does not activate the basal level of apoptosis dramatically (Schnepp et al., 2004b).

Regulation of genome instability

Peripheral lymphocytes from MEN1 patients display increased chromosome breakage (Gustavson et al., 1983,Scappaticci et al., 1991). Moreover, these lymphocytes undergo extensive chromosomal breakage after treatment with diepoxybutane, an agent crosslinking double stranded DNA (Itakura et al., 2000,Tomassetti et al., 1995). A genome-wide screening of loss of heterozygosity of MEN1 on pancreatic tumors indicates that the tumor cells fail to maintain DNA integrity and chromosomal stability (Hessman et al., 2001). However, no obvious chromosomal instability was observed in the islet cells in which *Men1* was lost (Scacheri et al., 2004). Thus, it is likely that *Men1* probably plays an important role in maintenance of genomic stability when the cells are under stress (Itakura et al., 2000).

Replication protein A2 (RPA2), a subunit of a trimeric protein binding to single stranded DNA, has been shown to interact with menin (Sukhodolets et al., 2003). RPA is involved in DNA replication, DNA repair, DNA recombination and gene transcription. Because RPA is crucial in DNA repair and in ATR-mediated sensing of DNA damage (Zou and Elledge, 2003), the interaction between menin and RPA2 suggest a potential mechanism for menin in DNA repair. Menin also interacts with FancD2, a protein involved in a BRCA1-mediated DNA repair pathway (Garcia-Higuera et al., 2001), and the interaction between menin and FancD2 is enhanced by γ-irradiation (Jin et al., 2003). Furthermore, targeted disruption of *Men1* increased sensitivity to DNA damage induced by an intra-stranded DNA crosslinking agent (Jin et al., 2003). Consistent with this observation, FancD2 also associates with chromatin (Wang et al., 2004). Recently, distribution of menin in cells has been shown to be regulated by UV irradiation (Farley et al., 2006). Thus, these reports suggest a crucial role for menin in repair of DNA damage in concert with RPA2 and FancD2. Menin also functionally interacts with ASK (Schnepp et al., 2004a), which plays a crucial role in response to DNA damage. Therefore, menin may participate in DNA repair in part by modulating the activity of Cdc7/ASK and/or transcription of other DNA repair-related genes.

Fruit flies with the disrupted *Men*1 homologous gene are viable, but are hypersensitive to ionizing irradiation and DNA-crosslinking agents (Bale, 2004). Consistent with this observation, menin has been found to regulate stress responses including heat shock (Papaconstantinou et al., 2005). These results further support that menin plays an important role in maintaining genome stability and cellular integrity. The precise role of menin in DNA repair and its mechanisms of action remain to be elucidated. Linking menin to RPA2, FancD2, hTERT, and cdc7/ASK points to an interesting direction for elucidating how menin participates in maintenance of genome stability.

Suppression of the MEN 1 development by menin

Menin represses cell proliferation, potentiates apoptosis (Sayo et al., 2002,Schnepp et al., 2004b), suppresses growth of transplanted tumors (Kim et al., 1999), and maintain the genome stability in culture cells (Itakura et al., 2000,Hessman et al., 2001,Jin et al., 2003,Scappaticci et al., 1992,Scappaticci et al., 1991). However, it is not yet clear whether these tumor-suppressing roles in culture cells are crucial for suppressing the development of MEN1. Hence, it is important to determine whether various MEN1 disease-related menin mutations compromise menin's various tumor-suppressing functions in both cell culture and mouse models.

Mutations in *Men1*, together with functional perturbations of other oncogenes and tumor suppressor genes, may orchestrate the development of the MEN1 tumors. *Men1* mutation is a relatively early event in MEN1 tumorigenesis since MEN1 tumors arise in a later stage following hyperplasia in human MEN1 patients and MEN1 mice (Crabtree et al., 2001). p53 is mutated in atypical carcinoids and hormone-secreting tumors that also overexpress Bcl-2

(Schnirer et al., 2003). However, it is unclear whether p53 and Bcl-2 are mutated in the MEN1 tumors. If so, it is important to determine whether the mutations take place prior to LOH of *Men1*. Thus, temporary molecular changes in various common oncogenes and tumor suppressor genes, in reference to the LOH of *Men1* during the MEN1 tumor development, should be further investigated. These studies should provide a temporary molecular order of mutations in various oncogenes and tumor suppressor genes, during the MEN1 tumor development.

Tumors in MEN1 patients preferentially develop in the endocrine organs (Marx et al., 1999a). Although the underlying mechanism is still poorly understood, several hints regarding their preference for endocrine organs exist. For example, like MEN1, patients with inherited multiple endocrine neoplasia type 2 (MEN2) also develop tumors in endocrine organs, primarily in the thyroid gland and the parathyroid gland (Eng, 1999); the parathyroid gland tumor is the most common tumor in MEN1 patients. In MEN2 patients, c-ret, a cell surface protein tyrosine kinase receptor for GDNF (glial cell-derived neurotrophic factor), is mutated, resulting in a constitutively active tyrosine kinase receptor (Eng, 1999), and triggers tumorigenesis in MEN2 patients. The constitutively active receptor induces cell proliferation and activation of the AKT pathway even in the absence of its ligand, GNDF. It is noteworthy that c-ret is highly expressed in neuroganglias and neuroendocrine tissues (Santoro et al., 1999). Thus, it is possible that the tumor-promoting effects of normal c-retin endocrine organs could be antagonized by menin's tumor-suppressing function. If this is the case, breeding mice expressing the oncogenic *c-ret* in endocrine cells (Cranston and Ponder, 2003) with mice with the $Men1^{+/-}$ genotype will accelerate the tumorigenesis in the endocrine organs.

Other evidence also exists that may explain why there is a high incidence of tumor development in the endocrine organs of MEN1 patients. Menin may cooperate with endocrine organ-specific factors and suppress cell proliferation, induce apoptosis, or regulate other genes functioning at crucial steps of tumorigenesis. Furthermore, it is also likely that menin transcriptionally regulates a subset of target genes in a tissue-specific manner to suppress tumorigenesis.

Perspectives

Numerous studies suggest a crucial role for menin in regulation of gene transcription, cell proliferation, apoptosis and genome stability. However, the precise underlying mechanisms for these functions remain to be elucidated. For instance, it is not clear whether transcriptional regulation is the most important means by which menin suppresses the MEN1 tumor development. How does menin precisely upregulate a group of genes, including $p27^{kip1}$, $p18^{ink4c}$, *caspase 8* and *Hoxc8*, but downregulates *hTERT*, cyclin D1 and CDK4? Are these menin target genes altered in MEN1 tumors? How does menin precisely regulate histone modifications and change chromatin structure? Is it possible to explore specific effect of menin inhibition on enhancing pancreatic β cell proliferation to ameliorate diabetes? Answers to these questions should not only provide novel insights into treating MEN1 syndrome and other related tumors, but also uncover the novel molecular circuitry to modulate function of multiple endocrine cells including pancreatic β cells.

ACKNOWLEDGMENTS

This work is in part supported by an American Cancer Society Research Scholar award and NIH grants (R01 CA100912 and CA113962). We thank Robert Schnepp, Drs. Alan Diehl and Zhao-yuan Hou for stimulating discussions and critically reading of the manuscript. We also thank Ms. Lauren Meade for excellent assistance in preparing the figures.

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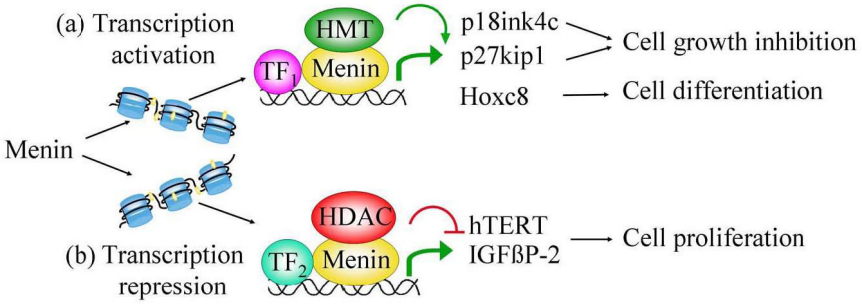

Figure 1.

A schematic model explaining how menin regulates gene transcription. (a) Menin and a hypothetically specific DNA binding protein (TF_1), together with transcription-activating histone methyltransferases (HMTs), such as MLL or MLL2, target to the loci of *p18*^{ink4c}, *p27*^{kip1} and *Hoxc8* genes in chromatin. This HMT-containing complex methylates lysine 4 on histone H3, and changes chromatin structure and subsequently activates gene transcription. Activation of *p18*^{ink4c}, *p27*^{kip1} and *Hoxc8* genes leads to cell growth inhibition or cell differentiation. (b) Menin and a hypothetically specific DNA binding protein (TF_2), together with a histone deacetylase (HDAC), may target the loci of menin target genes such as *hTERT* and *IGFBP-2*, to remove the acetyl group on histones and thus repress the target gene transcription. Inhibition of *hTERT* and *IGFBP-2* may result in reduced cell proliferation and maintenance of genomic stability. Interaction of menin and HDACs in regulating endogenous genes remains to be determined.

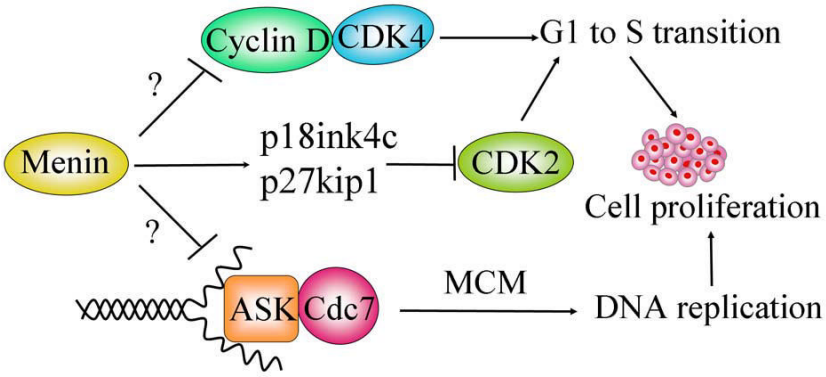

Figure 2.

A schematic model showing how menin regulates cell proliferation via distinct mechanisms. Menin represses expression of Cyclin D1 and D3, which form an active kinase with cyclin-dependent kinase (CDK4), promoting cell cycle progression from G1 to S phase. Menin also upregulates the expression of $p18^{ink4c}$ and $p27^{kip1}$, and represses cyclin-dependent kinase 2 (CDK2) activity and G0/1 to S transition. Cdc7/ activator of S-phase kinase (ASK) complex possesses protein kinase activity phosphorylating mini-chromosome maintenance (MCM) protein, and is essential for DNA replication and S phase progression. Menin interacts with ASK and inhibits ASK-induced cell proliferation. It is likely that, through either transcriptional or post-transcriptional regulation, menin represses CDK4/Cyclin D, CDK2/Cyclin A or E, and Cdc7/ASK, leading to repression of cell cycle transition from G1 to S phase or progression through the S phase.

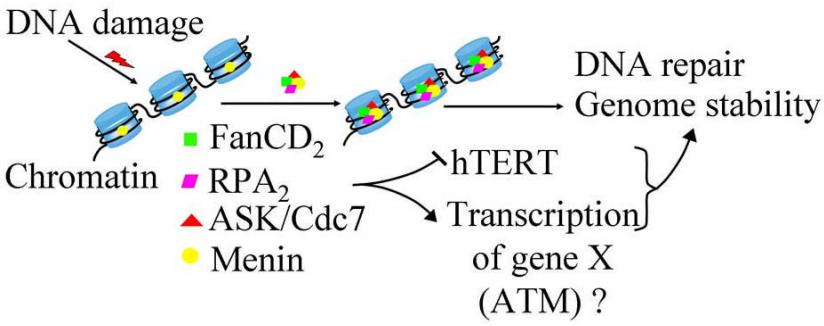

Figure 3.

A model for regulation of genome stability by menin via association with various nuclear proteins. Menin associates with chromatin and, upon DNA damage induced by ionizing irradiation or DNA crosslinking, increases its affinity with FancD2, a protein involved in DNA repair and genome stability. In addition, menin also interacts with Cdc7/ASK (activator of S-phase kinase), which is also involved in DNA replication and repair, and with monomeric RPA2 (replication protein A2). DNA damage may increase menin's affinity with these interacting proteins and thus form a supper complex on the chromatin. Although menin alone interacts with each of the above proteins, it remains to be shown whether it forms a supper complex with the other four proteins simultaneously. Menin also represses hTERT (human telomere reverse transcriptase) and thus may protect genome stability indirectly. Because menin's prominent role in regulating gene transcription, it is also possible some of its target genes are involved in maintaining genome stability (Scacheri et al., 2006).