

## REVIEW

# The role of menin in bone pathology

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## Abstract

Multiple endocrine neoplasia type 1 (MEN1) is the most common cause of hereditary primary hyperparathyroidism (PHPT). Bone disorders are considered one of the key symptoms in PHPT present with the significant reduction in bone mineral density and low-energy fractures. Previously, these bone disorders were believed to be caused solely by the increase in the level of parathyroid hormone and its subsequent effect on bone resorption. The current paradigm, however, states that the mutations in the menin gene, which cause the development of MEN1, can also affect the metabolism of the cells of the osteoid lineage. This review analyzes both the proven and the potential intracellular mechanisms through which menin can affect bone metabolism.

### Key Words

- ▶ menin
- ▶ men 1 protein
- ▶ osteoporosis
- ▶ multiple endocrine neoplasia type 1

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## Introduction

Menin is a highly conserved protein encoded by the ubiquitously expressed *MEN1* gene. It partakes in the regulation of various intracellular processes, including transcription, maintaining genome stability, proliferation and intracellular signaling. Mutations in the menin gene cause the development of multiple endocrine neoplasia type 1 (MEN1) syndrome. This autosomal dominant disease is characterized by the formation of multiple tumors, predominantly located in the endocrine glands. In MEN1, lesions of parathyroid glands have the highest penetrance; they manifest with primary hyperparathyroidism (PHPT). Conversely, PHPT is a powerful etiological factor that can lead to bone disorders including the significant decrease in bone mineral density (BMD) followed by multiple low-energy fractures. In contrast to sporadic PHPT, bone disorders in patients with PHPT in multiple endocrine neoplasias (PHPT/MEN1) are more severe. The higher severity of bone and mineral disorders in patients with menin gene mutations has been clinically proven (1): bone mineral densities of the lumbar spine and at the femoral neck observed in PHPT/MEN1 patients are significantly lower compared to those of sporadic PHPT patients. Bone demineralization is observed in up to 77.8% of

reaches osteoporotic levels (1, 2, 3). Additionally, the bone formation rate in PHPT/MEN1 patients after

parathyroidectomy is significantly lower, than for sporadic PHPT (4).

Several differences exist in the structure of the changes as well. In PHPT/MEN1, both cortical and trabecular bone tissues are demineralized, while in sporadic PHPT, primarily cortical bone tissue is affected (5).

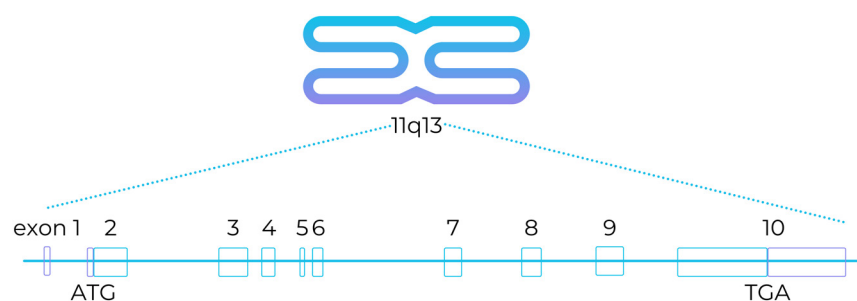
At the same time, the quality of the clinical studies that have been published to date is lacking, mainly due to the orphan nature of the disease and the variety of possible comorbidities. An unambiguous interpretation of these studies is not possible, which necessitates the generation of a suitable experimental model.

In the absence of human cell-based models, mice were used to study the role of menin in bone metabolism *in vitro* and *in vivo*. A number of recent works have shown that menin is directly involved in the regulation of osteoblastogenesis, differentiation and functioning of osteoblasts, and in the osteocyte-osteoclast interaction. It has been shown that the effects of menin can be mediated by several potential mechanisms, such as the interactions with the Runx2 transcription factor, molecules involved in the  $\beta$ -catenin pathway, BMP, and Smad3, Smad1/5 and JunD signaling molecules (6, 7, 8). Through these, menin affects osteoclastogenesis, as well as the formation and functioning of osteoblasts.

Bone remodeling is a process that takes place throughout a person's life. It involves the destruction of old bone tissue and its replacement with a newly formed bone matrix. The main cells involved in bone remodeling are osteoblasts (bone-forming cells) and osteoclasts (cells that degrade bone tissue). The combined action of these cells leads to the successive resorption of the old bone and the formation of a new one (9). The existing findings point to the important role of menin in bone remodeling, which is a continuous dynamic interaction of the bone-forming cells (osteoblasts) and the bone-resorbing cells (osteoclasts). However, models that use transformed cell lines and/or *MEN1*-knockout animals cannot fully reproduce the extremely variable clinical presentation of *MEN1* in humans and cannot account for the possible influence of individual genetic and epigenetic changes on the course of bone disorders. An *in vitro* isogenic model based on *MEN1* patients' cells, generated using cell reprogramming and genome editing, would present a promising approach to uncover the mechanisms underlying the role of menin in bone metabolism considering individual genetic and epigenetic features. In addition to the benefits provided by personalization, this model can be used to identify specific therapeutic targets and develop modern effective therapeutic algorithms for *MEN1*. However, differentiation of induced pluripotent stem cells, mesenchymal stem cells (MSCs) or fibroblasts into osteoblasts is also a non-trivial task. In particular, stem cells can form teratomas, their collection is a traumatic procedure, and they also lose their ability to differentiate after five passages. Dermal fibroblasts are capable to undergo 15 passages, but they are differentiated cells, and it is challenging to redirect them toward specific lineages (10).

### Structure of menin

Menin is a highly conserved protein encoded by the *MEN1* gene, which is located on chromosome 11 and consists of 10 exons that encode 610 amino acid residues.



**Figure 1**  
Schematic chromosomal localization and structure of *MEN1*. Blue and violet boxes indicate the coding and non-coding regions of exons, respectively (11).

The schematic structure of the coding regions of *MEN1* is shown in Fig. 1.

It has no homology domains with known consensus sequences, which makes the study of its intracellular interactions problematic. Some insights were gained after the structure of menin was determined.

It was established that the C-terminus of menin has two functionally independent nuclear localization signals (NLS) which provide its predominant nuclear localization. Additionally, C-terminus allows menin to bind to the dsDNA directly, with no specific binding site required. NLS is necessary to form such a bond. It has also been suggested that menin may specifically bind to some DNA regions via two leucine zipper motifs (12).

The N-terminus of menin contains five motifs usually found in GTPases and GTP-binding proteins. At the same time, the affinity of menin for GTP is relatively low, and it displays GTPase activity only in presence of nm23 nucleoside diphosphate kinase (12).

Menin is expressed in many tissues; however, its function in an adult organism is tissue-specific. In fact, the effects of menin in different organs can be directly opposite. For example, it acts as a tumor suppressor in endocrine tissues but induces proliferation in leukopoiesis. The reasons for this are ambiguous, as to date there is no clear understanding of the various intracellular cascades menin participates in (12).

### Menin in the development of bone disorders in *MEN1* patients

Parathyroid lesions in *MEN1* manifest with PHPT, usually followed by hypersecretion of parathyroid hormone and hypercalcemia. Development of complications in key target organs is characteristic both for sporadic and hereditary PHPT. That includes the reduction of the BMD up to the development of osteoporosis and osteitis fibrosa cystica (1).

A study performed in the Russian population shows that skeletal complications arise in 87% of patients with

sporadic hyperparathyroidism (13). In PHPT/MEN1, BMD is decreased in 58–72% of the cases (5, 14).

The clinical course of bone disease in PHPT/MEN1 patients differs from that in sporadic PHPT/MEN1. For instance, BMD at the time of diagnosis is usually lower in PHPT/MEN1 (2, 15). Additionally, in osteoporosis caused by sporadic hyperparathyroidism, the parathyroidectomy is followed by a relatively rapid gain in bone mass; for that reason, anti-osteoporotic drugs are not prescribed to these patients for the first few years after the surgery (16). Parathyroidectomy in PHPT/MEN1 patients leads to a significantly slower bone mass gain (4).

There is no definite explanation for these differences. In part, they may be caused by the poor methodology of research (e.g. the use of surrogate endpoints instead of the immediate BMD values) (4). Additional BMD loss in PHPT/MEN1 may be caused by the components associated with the syndrome (i.e. hormone-producing neuroendocrine tumors of the gastrointestinal tract) (4, 17). Slower bone mass gain after surgery may be caused by a greater extent of surgery and a high incidence of postoperative hypoparathyroidism (4).

The results of fundamental and clinical research suggest that heterozygous deletions of *MEN1* affect bone metabolism. The review of the main intracellular pathways in which menin participates is presented below. Disruption of these pathways can result in bone metabolism disorders in MEN1 patients.

According to the Knudson two-hit hypothesis, inactivation of both alleles of the gene is required for carcinogenesis: the first mutation is inherited, the second is acquired during lifetime (18). Haploinsufficiency is not necessarily accompanied by disruption of cellular functions. For instance, the expression of menin and its messenger molecules (mixed lineage leukemia (MLL), p27 and p18) in the pancreas and pituitary gland does not differ in MEN1+/- and WT mice (19).

At the same time, in 2001 it was already shown by Crabtree *et al.* (20) that homozygotic inactivation of menin in mice results in embryo loss, with some fetuses developing gross malformations of the skull and facial bones. *MEN1* deletion can result in perinatal death, development of cleft lip or other cranial malformations associated with disorders of bone mineralization; additionally, menin deficiency leads to anomalies of rib development (20, 21, 22). Since the skull and facial bones are formed by intramembranous ossification, further questions arose regarding the role of menin in the differentiation of multipotent MSCs into osteoblast lineage cells and in the differentiation of osteoblasts.

Later experiments used mice with MEN1 and have repeatedly confirmed that menin participates in the regulation of normal osteogenesis and bone mass gain (7). Trabecular bone volume and cortical bone thickness are significantly lower in osteoblast *Men1* knockout mice (Men1-/-) compared to menin overexpression mice (7). Men1-/- mice have fewer osteoblasts and osteoclasts, lower mineral apposition rate, and a higher number of osteocytes (7). At the same time, transgenic mice with hyperexpression of menin display the opposite phenotype with an increased number of osteoblasts, mineral apposition rate and bone mass in comparison with WT mice (7).

In agreement with a study by Kanazava *et al.*, Liu *et al.* observed a decrease in bone formation in 12-month-old female mice. However, in middle-aged mice, the authors show that selective *Men1* deletion in the osteoblastic lineage in three distinct mouse lines does not alter bone formation. In contrast to Kanazawa *et al.*, in primary uncommitted mesenchymal stromal cells of young mice with an efficient deletion of *Men1*, there were no differences in osteoblast proliferation and their differentiation potential. No major difference was evident in either Smad1/5/8 phosphorylation or Smad3 phosphorylation upon BMP2 and TGFβ stimulation, respectively. These differences may be related to different methodologies in these studies as well as that primary cells analyzed by authors were received from the different aged mice (8).

This may be in part due to the disruption of intracellular interactions between osteocytes and osteoblasts. Changes characteristic for menin haploinsufficiency manifest in the bone tissue even if the mutation only affects osteocytes without disrupting the function of menin in osteoblasts. Presumably, this is due to a change in the osteocytic expression of the C-X-C motif chemokine 10, a soluble mediator which promotes osteoclastogenesis (23).

## Menin and transcription factors

Menin can interact with multiple regulatory molecules, including JunD, NF-κB, Smad3 and MLL (12, 24, 25). It is involved in various cellular processes such as transcriptional regulation, DNA replication and repair and cell cycle (26). Although much is known about the effects of menin in different tissues, the data on the role of menin in osteogenic cells are limited.

## Menin and NF $\kappa$ B

NF $\kappa$ B is a transcription factor that functions as an important regulator of apoptosis and proto-oncogene. In intact cells, NF $\kappa$ B is predominantly localized in the cytoplasm in an inactive form, bound to the inhibitor protein I $\kappa$ B. In response to certain stimuli (ionizing radiation, mitogens, growth factors, cytokines and others) I $\kappa$ B is phosphorylated, and NF $\kappa$ B is translocated into the nucleus (26, 27).

NF $\kappa$ B complexes are represented by homo- and heterodimers of transcription factors p50, p52, p65 (RelA), c-Rel and RelB. p50, p52 and p65 directly interact with menin *in vivo* and *in vitro*. p65 activates transcription in NF $\kappa$ B-associated sites, while p50 and p52 suppress transcription by competitively binding to these respective sites (27). Menin also suppresses p65-mediated transcriptional activation of NF $\kappa$ B sites in a dose-dependent manner; in this case, menin insufficiency can lead to oncogenesis (27).

Receptor activator of NF $\kappa$ B and its ligand (RANK/RANKL) system presents a major regulatory pathway of bone metabolism. RANK/RANKL are responsible for the differentiation and maturation of osteoclasts, which are required for the bone resorption phase of bone remodeling (28). Thus, mutations in the menin gene may lead to transcriptional activation of NF $\kappa$ B-associated sites; that can, presumably, mimic the intensive functioning of the RANK/RANKL system, increasing bone resorption.

## Menin and JunD

JunD is a transcription factor from the activator protein 1 family. It is involved in the suppression of cell proliferation (29, 30). Menin and JunD interact via the C-terminus of menin and the transactivation domain located at the N-terminus of JunD.

Menin inhibits JunD-induced transcription suppression in two ways. First, it interacts with histone deacetylases, mSin3A in particular (30, 31). Secondly, it inhibits c-Jun N-terminal kinase, which activates JunD by phosphorylation in the S90, S100 and T117 positions (32, 33).

The fact that menin, an oncosuppressor, inhibits the activity of another proliferation inhibitor – JunD – may seem puzzling. However, JunD acts as a proliferation inhibitor only in complex with menin; without it, JunD activates proliferation instead. That being said, JunD mostly exists in the form of a JunD-menin complex (34, 35).

Two isoforms of JunD are currently known: the full-length JunD-FL and the truncated  $\Delta$ JunD. Transcription of these isoforms begins at two different start codons of the same mRNA. Menin suppresses the transcriptional activity of JunD-FL, but not  $\Delta$ JunD, so only the full-length isoform is a functional partner of menin (35).

Little is known about the specific endogenous targets affected during the suppression of cell proliferation by the menin/JunD complex. In bone metabolism, JunD activates osteoblast differentiation and their subsequent mineralization. Excessive expression of menin suppresses these processes (and inhibits alkaline phosphatase (ALP) activity) (36). Naito *et al.* studied the menin-JunD interactions in regard to osteoblast differentiation using the MC3T3-E1 mouse osteoblastic cell line. Immunoblotting was used to measure JunD expression, which gradually increased during osteoblast differentiation. Stable expression of JunD enhanced the expression of differentiation markers, Runx2, type 1 collagen, and increased osteocalcin and alkaline phosphatase activity and mineralization. In MC3T3-E1 cells with artificially reduced menin expression, JunD levels were significantly increased. JunD overexpression increased the transcriptional activity of AP-1, while inhibition of menin expression reduced it. In turn, menin suppressed JunD-induced transcription activity and inhibited ALP activity in the MC3T3-E1 osteoblasts (36). Kaji *et al.*, who also showed that menin suppresses the maturation of osteoblasts (6), obtained similar data. This is confirmed by the fact that JunD-knockout mice have higher bone mass and trabecular number, and display higher osteoblast activity (37). Thus, according to the literature data, menin suppresses osteoblast maturation, in part, by inhibiting the anabolic effect of JunD on the bone tissue.

## Menin and Runx2

Runx2 is a key transcription factor for osteoid cells, expressed in MSCs, osteoblasts and chondrocytes. Runx2 is required for osteoblast differentiation and proliferation of osteoprogenitor cells (where it can work in conjunction with the Wnt signaling pathway). In addition, Runx2 induces the proliferation of MSCs and their transition into osteoid cells (37, 38).

Bone morphogenetic protein 2 (BMP-2) belongs to the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily, which plays a key role in osteoblast differentiation. Inactivation of menin by antisense oligonucleotide sequences suppresses the BMP-induced ALP activity and the expression of type 1 collagen, Runx2 and osteocalcin. Adipogenesis and

chondrogenesis, in which BMP-2 is also involved, remain unaffected (39).

Menin/Runx2 are important not only during the early phases of osteoblast differentiation; they participate in the inhibition of late stages of the process as well. Menin interacts with Runx2 in MSCs, but not in differentiated osteoblasts. Menin suppression of Runx2-induced transcription leads to the inhibition of late stages of osteoblast differentiation (40).

### Menin and histone modifiers

Menin is predominantly a nuclear protein. It can act as a scaffold protein, regulating gene transcription by coordinating various chromatin-associated proteins. For example, menin can interact with MLL (41) and enhancer of zeste homolog 2 (EZH2) (42) histone methyltransferases and histone deacetylases (43).

### Menin and cyclin-dependent kinase inhibitors

Menin can directly regulate the expression of the cyclin-dependent kinase inhibitor (CDKI) genes *Cdkn1b* and *Cdkn2c*, which encode p27Kip1 and p18Ink4c, respectively (43, 44, 45, 46). P27Kip1 and p18Ink4c, in turn, play a key role in suppressing proliferation (47). To achieve this effect, menin attracts MLL to the promoter and coding regions of *Cdkn1b* and *Cdkn2c*, where MLL catalyzes histone H3 lysine 4 (H3K4) methylation, stimulating the transcription of these genes (43, 44, 45, 46). In mouse embryonic fibroblasts, loss of both MLL and menin leads to reduced expression of p27Kip1 and p18Ink4c and to reduced proliferative activity (43, 44, 45). Here, the role of tumor suppressor is played by CDKI.

*Cdkn1b*<sup>-/-</sup> mice display an increase in proliferative activity of osteoprogenitor cells with the formation of numerous large colonies of osteoblasts. At the same time, intensive proliferation is not followed by differentiation and tumor growth (47, 48). This suggests that menin mutations can also lead to increased proliferation of osteoid cells, but the experiments proving that *Cdkn1b* is indeed involved in menin function in bone are missing.

### Menin and homeobox genes

The nature of interaction between menin and homeobox genes (Hox genes, highly concerned DNA sequences required during prenatal development) is ambiguous.

On the one hand, menin acts as an oncogenic transcription coregulator of Hox genes in leukemia, binding to various homeobox genes during hematopoiesis and myeloid transformation (49). On the other hand, menin can also interact with Hox genes as a tumor suppressor, for example, in parathyroid tumors (49, 50). Therefore, the direction of this interaction depends on the specific cell type in question.

Hox genes control the spatial patterning of the developing embryo through multicomponent transcription factors. These are not only limited to transcriptases but also include transcription coregulators. Since coregulators can inhibit transcription as well as promote it, Hox genes simultaneously inhibit the expression of 'old' morphogenetic genes and promote the expression of the 'new' ones. Hox genes are important for the development of the head-tail axis in animals with bilateral symmetry, including humans, and skeleton development as a part of it. These genes retain their function in the adult organism. For instance, *Hox11* is expressed in the MSCs and in the areas where bone healing occurs (i.e. at fracture sites) (51). In osteoblast culture, upregulation of Msh homeobox 2 corresponds to an increased cell proliferation, migration and osteogenic differentiation (52). To date, no studies on the interaction between menin and these homeobox genes have been published.

### Menin, pleiotrophin and Polycomb group proteins

Pleiotrophin (PTN) is a growth factor, which is richly expressed in many solid carcinomas (53). In lung epithelial cells, menin inhibits the transcription of the *PTN* gene by recruiting Polycomb group proteins (PcG) and inducing histone H3 lysine 27 (H3K27) methylation (53, 54). In this case, loss of menin increases PTN expression (53, 54, 55).

PcG genes encode a family of proteins responsible for chromatin remodeling, in particular, the polycomb repressive complex 2, which contains the EZH2 methyltransferase and its regulatory protein, SUZ12 (56, 57). EZH2 is a chromatin-associated protein with a conserved SET domain, which performs H3K27 methylation. This, in turn, leads to chromatin condensation and inhibition of transcription (58).

Menin, therefore, binds to the promoter region of the *PTN* gene and, together with EZH2, performs H3K27 methylation, suppresses *PTN* and inhibits proliferation (54). Without menin, EZH2 and SUZ12 cannot bind to *PTN*, and the intensity of H3K27 methylation is reduced.

In osteogenesis, EZH2 plays an ambiguous role: it inhibits the differentiation of MSCs into the osteogenic lineage, and at the same time enhances the proliferation of osteoprogenitor cells (59, 60). Given that menin regulates EZH2, it can be assumed that this methyltransferase is one of the molecules responsible for the development of bone disease in *MEN1* mutation cases.

### Menin and HDAC3

Cyclin B2 is one of the molecules that control the G2/M cell cycle transition. It is encoded by the *Ccnb2* gene. By binding to the promoter region of *Ccnb2*, menin inhibits its transcription. Additionally, it interacts with histone deacetylase 3 (HDAC3), which is found in the same locus; that reduces the intensity of H3 acetylation. Cultured cells with menin mutations analogous to those found in *MEN1* patients display no inhibition of histone H3 acetylation of the *Ccnb2* locus and, therefore, no inhibition of *Ccnb2* transcription. Since G2/M transition is facilitated by cyclin B2, it occurs faster in the cells with menin mutations (43).

Apparently, *Ccnb2* also participates in bone metabolism: it has been shown to have a richer expression in bone calluses of osteoporotic mice compared to the WT mice (60). It can be assumed that patients with osteoporosis in *MEN1* will display similar features; perhaps, they would be even more pronounced due to the lack of functional menin.

### Menin and nuclear receptors

The nuclear receptor PPAR $\gamma$  is predominantly expressed in adipose tissue, the large intestine and macrophages, but can be found in other tissues as well. Regulation of carbohydrate and lipid metabolism and activation of M2 macrophages are considered its classical effects. Menin interacts with the activation domain of PPAR $\gamma$  in a ligand-independent manner and uses the H3K4 methyltransferase to increase the expression of the receptor. PPAR $\gamma$  inhibition is believed to trigger the development of lipomas in *MEN1* patients (61).

PPAR $\gamma$  is also expressed in bone tissue (62), where it is responsible for the conversion of osteoblasts into adipocyte-like cells (63, 64) and their differentiation (65). Different PPAR $\gamma$  ligands can affect bone metabolism; for example, rosiglitazone inhibits the expression of Runx2/Cba1, the transcription factor required for osteoblast differentiation and synthesis of osteocalcin (66). In animals, this leads to loss of BMD in various parts of the skeleton (67). Endogenous

activators of PPAR $\gamma$  display similar properties regarding bone formation (68). Also, according to some research, PPAR $\gamma$  is able to induce apoptosis in cells of the osteogenic line (69). Moreover, PPAR $\gamma$  activation inhibits bone resorption: as such, ciglitazone inhibits osteoclastogenesis in a dose-dependent manner, apparently due to the effect on osteoprotegerin (70). Therefore, it can be argued that *MEN1* mutations may lead to increased osteoclastogenic activity through PPAR $\gamma$  inhibition.

WT menin also directly promotes the transcription of vitamin D receptor (VDR) and estrogen receptor alpha (ER $\alpha$ ), two other steroid receptors crucial for bone formation and remodeling (71). VDR, in turn, regulates the activity of p27Kip1 and p18Ink4c (their potential role in bone metabolism has been discussed earlier) (72, 73). However, despite the undoubted importance of vitamin D in bone metabolism, no data have been published regarding the effect of *MEN1* mutations on the implementation of VDR-mediated and ER $\alpha$ -mediated effects.

### Menin and intracellular signaling pathways

Menin can inhibit cell proliferation through several intracellular signaling pathways.

#### Menin and the transforming growth factor $\beta$ signaling pathway

TGF- $\beta$  is richly expressed in the bone matrix; there, it is stored in an inactive form and can be activated by the cells of the bone microenvironment (74). The TGF- $\beta$ -mediated pathway inhibits the proliferative and transcriptional activity of the cell through specific Smad signal transducers, such as Smad2, Smad3 and others. Receptor-mediated phosphorylation of Smad2 or Smad3 allows them to bind to Smad4. The resulting complex translocates into the nucleus, where it activates the transcription of target genes (75). Here, menin activates TGF- $\beta$  and Smad3-induced transcription by inhibiting the binding of Smad3 to the transcription regulatory binding sites of the DNA (76). Menin inactivation inhibits the TGF- $\beta$ -dependent signaling pathway, which, in turn, leads to excessive cell proliferation and tumor growth. Smad3 appears to be important for osteoclast formation; its inactivation inhibits osteoclastogenesis (77).

Mice with menin deletions in MSCs or osteoblast progenitor cells develop ossifying fibromas of the jaw. These tumors are characterized by lower BMD, higher bone resorption and a more pronounced stromal component.

The stromal cells isolated from these masses display higher TGF- $\beta$  expression (78).

Interestingly, TGF- $\beta$  increased ALP expression and osteoblast mineralization in a study by Sowa *et al.*, even though it typically inhibits osteoblast differentiation (79).

Additionally, it is through Smad that menin drives MSC differentiation toward the osteogenic or myogenic lineage. BMP-2 induces the ectopic formation of bone and cartilage in nonskeletal tissues. It is crucial for bone formation and osteoblast differentiation (74). In response to BMP-2, menin increases Smad1/5 transcriptional activity. This directs the differentiation of MSCs into the osteoblast lineage. Moreover, when influenced by TGF- $\beta$ 1, menin increases the inhibition of Smad3/TGF- $\beta$ 1-mediated myogenic differentiation of MSCs. In case of menin deficiency, MSCs primarily differentiate into the myogenic lineage (80). Menin is also a positive regulator of miR-26a, a miRNA that inhibits SMAD1 expression during the osteoblastic differentiation of MSCs (81).

### Menin and Wnt/ $\beta$ -catenin signaling pathway

The classic Wnt/ $\beta$ -catenin signaling pathway plays a crucial role in bone formation. It mediates the antiapoptotic effect

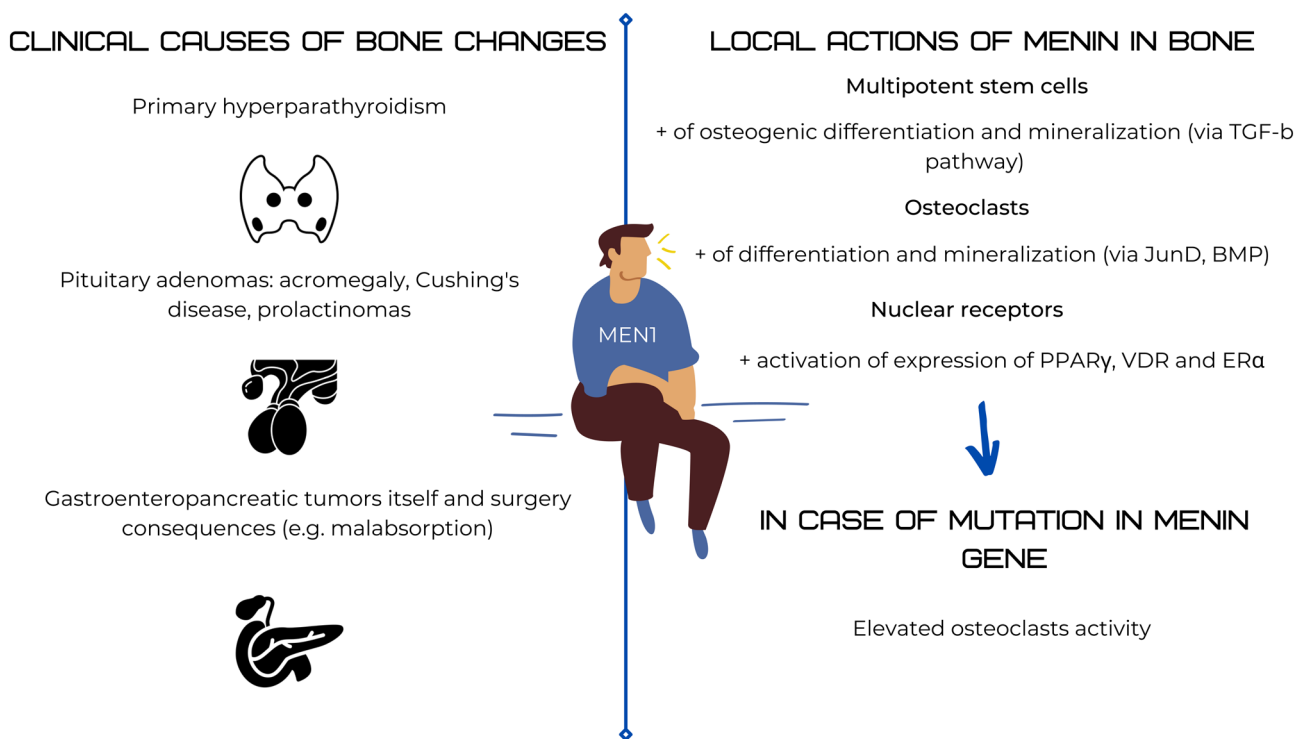
of parathyroid hormone on osteoblasts and participates in their BMP-induced differentiation (82, 83).

The C-terminus of menin directly interacts with  $\beta$ -catenin and activates its ubiquitin-mediated degradation. Additionally, menin suppresses the expression of  $\beta$ -catenin and its target genes, thus displaying an antiproliferative effect (84).

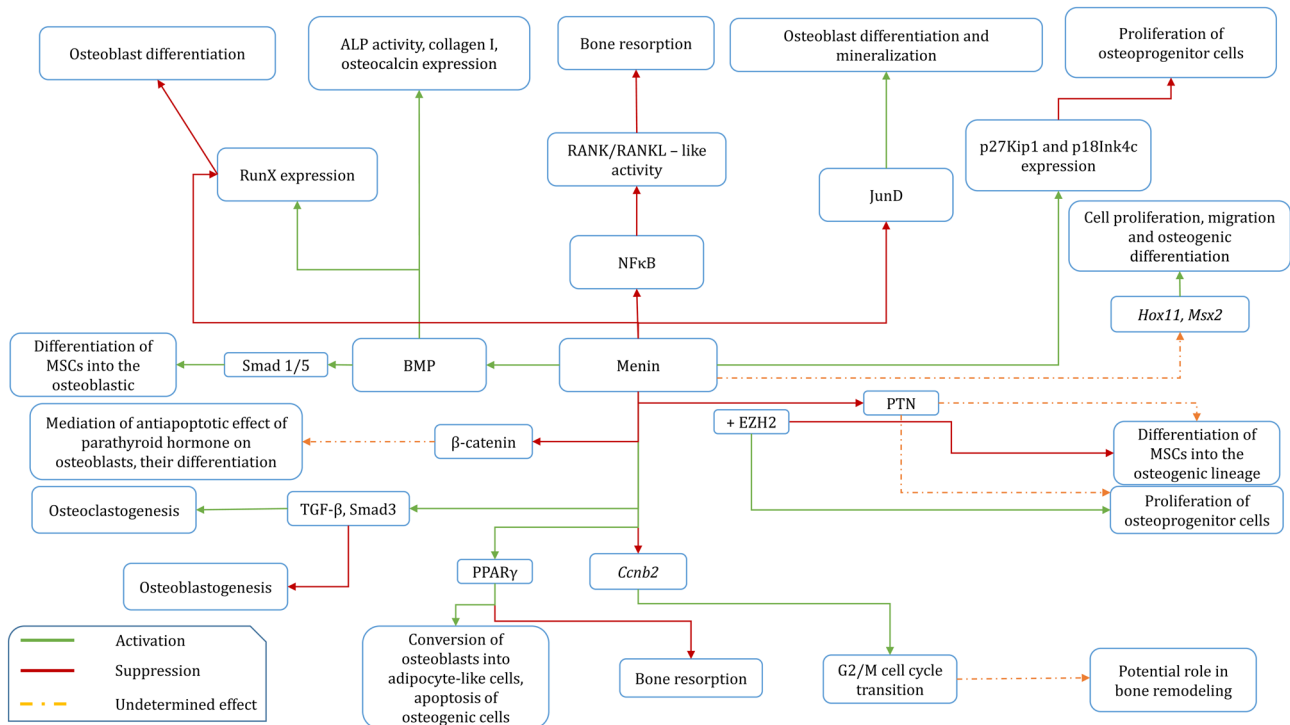
Administration of antisense nucleotides can inhibit menin, which prevents the BMP-2- and  $\beta$ -catenin-mediated increase in Runx2 and ALP expression. Thus, menin can affect BMP-induced osteoblast differentiation (85).

### Conclusion perspectives of studying the function of menin in bone tissue

The reviewed limited experimental data obtained in transformed mammalian cells suggest a general increase in osteoclast activity following *MEN1* mutations. Briefly, available information on the effect of menin on bone metabolism is shown in Figs 2 and 3. However, specific molecular mechanisms underlying the more severe PHPT-associated osteoporosis in *MEN1* patients have not yet been studied, despite the fact that this research could



**Figure 2**  
Schematic overview of menin and bone interactions.



**Figure 3**

Schematic overview of molecular interaction of menin in the aspect of bone metabolism. ALP, alkaline phosphatase; BMP, bone morphogenic protein; Ccnb2, cyclin B2 gene; EZH2, enhancer of zeste homolog 2; MSCs, multipotent stem cells; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; PTN, pleiotrophin; TGF- $\beta$ , transforming growth factor  $\beta$ .

potentially introduce a dramatic change to the existing therapeutic strategies.

The lack of sufficient knowledge in this area is explained by the difficulties in the generation of adequate *in vitro* models based on patients' cells. Manifestation age of MEN1 is highly variable, as are the spectrum and severity of clinical signs and symptoms. This is likely due to a combination of individual genetic and epigenetic factors. Additionally, the genetic reasons for such a variety of possible clinical manifestations are not limited to MEN1 mutations but are dictated by the genotype as a whole: mutated MEN1 is inherited along with a plethora of other gene combinations that can affect its role in different tissues.

Animal models are not sufficiently pure and detailed to study the role of menin in bone metabolism due to their multifactorial nature. A model based on human cells with MEN1 mutation seems to present an optimal solution for the development of new effective therapeutic approaches. Such a model should account for the individual features of the genome and would allow to pinpoint the changes introduced to osteogenic cell formation and functioning by MEN1 mutations.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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**Author contribution statement**

Gorbacheva A M, Eremkina A K, Goliusova D V, Krupinova J A and Mokrysheva N G contributed to the analysis of the literature data and to the writing of the manuscript.

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