THE ROLE OF NON-CODING RNAS IN THE PATHOGENESIS OF MULTIPLE ENDOCRINE NEOPLASIA SYNDROME TYPE 1

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Changes in the expression of non-coding ribonucleic acids (ncRNAs) takes part in the formation of various tumors. Multiple endocrine neoplasia syndrome type 1 (MEN1) is a rare autosomal dominant disease caused by mutations of the *MEN1* gene encoding the Menin protein. Syndrome is characterized by the occurrence of parathyroid tumors, gastroenteropancreatic neuroendocrine tumors, pituitary adenoma, as well as other endocrine and non-endocrine tumors. The mechanisms for the formation of MEN1-related tumors due to mutations in the *MEN1* gene are not . In the absence of mutations of the *MEN1* gene in patients with phenotypically similar features, this condition is regarded as a phenocopy of this syndrome. The cause of the combination of several MEN-1-related tumors in these patients remains unknown. The possible cause is that changes in the expression of ncRNAs affect the regulation of signaling pathways in which Menin participates and may contribute to the development of MEN-1-related tumors. The identification of even a small number of agents interacting with Menin makes a significant contribution to the improvement of knowledge about its pathophysiological influence and ways of developing tumors within the MEN-1 syndrome and its phenocopies.

KEYWORDS: multiple endocrine neoplasia syndrome type 1; menin; non-coding RNAs; microRNA; MEN1.

РОЛЬ НЕКОДИРУЮЩИХ РНК В ПАТОГЕНЕЗЕ СИНДРОМА МНОЖЕСТВЕННЫХ ЭНДОКРИННЫХ НЕОПЛАЗИЙ 1 ТИПА

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Изменение экспрессии некодирующих рибонуклеиновых кислот (нкРНК) играет роль в образовании различных опухолей. Синдром множественных эндокринных неоплазий 1 типа (МЭН-1) — редкое аутосомно-доминантное заболевание, обусловленное мутациями в гене *MEN1*, кодирующем белок менин. Синдром предрасполагает к развитию опухолей околощитовидных желез, нейроэндокринных опухолей желудочно-кишечного тракта, аденом гипофиза, а также других эндокринных и неэндокринных опухолей. Механизмы образования МЭН-1-ассоциированных опухолей вследствие мутаций в гене *MEN1* неясны. При отсутствии мутаций в гене *MEN1* у пациентов с фенотипически схожими чертами данное состояние расценивается как фенокопии этого синдрома. Причина сочетания нескольких МЭН-1-ассоциированных опухолей у таких пациентов остается неизвестной. Возможно, что изменения в экспрессии нкРНК влияют на регуляцию сигнальных путей, в которых принимает участие менин, и могут способствовать развитию МЭН-1-ассоциированных опухолей. Идентификация даже незначительного количества агентов, взаимодействующих с менином, вносит существенный вклад в повышение уровня знаний о его патофизиологическом влиянии и способах развития опухолей в рамках синдрома МЭН-1 и его фенокопий.

КЛЮЧЕВЫЕ СЛОВА: синдром множественных эндокринных неоплазий 1 типа; менин; некодирующие РНК; микроРНК; MEN1.

INTRODUCTION

Multiple endocrine neoplasia syndrome type 1

Multiple endocrine neoplasia syndrome type 1 (MEN-1) is a rare disease with an autosomal dominant pattern of inheritance. The syndrome is characterized by the development of a combination of parathyroid tumors (90%), gastrointestinal tumors (30–70%), and pituitary tumors (30-40%) [1]. Moreover, tumors in more than 20 other endocrine and non-endocrine tissues (including adrenal tumors — about 40% of cases) can develop in MEN-1 patients [1, 2]. In 1988, scientists from the Karolinska Institute in Stockholm and Uppsala University Hospital mapped the *MEN1* gene on the long arm of chromosome 11q13 [3], whose germinal mutations lead to the MEN-1 syndrome. The gene itself was discovered in 1997 [4]. The *MEN1* gene encodes the menin protein the functions of which will be discussed below. Today, more than 1,600 mutations of this gene have been described, where 23% are nonsense mutations, 20% — missense mutations, 41% — deletions and insertions with a frameshift, 6% — in-frame deletions and insertions, 9% — splicing mutations, and 1% — large deletions [5]. About 85% of cases are familial forms of the MEN-1 syndrome, while sporadic forms (one patient is identified in a previously unaffected family) are much less frequent (about 15% of cases) [5].

The reasons why the *MEN1* gene is a tumor suppressor can be explained by Knudson's « two-hit» hypothesis [6]. In 1971, A. Knudson proposed a hypothesis explaining the pattern of hereditary and sporadic forms of retinoblastoma.

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He suggested that two consecutive mutations must occur to trigger tumor growth in a cell: a germinal mutation followed by a somatic mutation. In a non-hereditary form, two mutations must occur in the same somatic cell, reducing the probability of such a coincidence; therefore sporadic retinoblastoma resulting from two somatic mutations occurs at a more mature age [6]. Loss of heterozygosity (LOH) on chromosome 11q13 in patients with germinal mutations of the *MEN1* gene is found in more than 90% of tumors, whereas in sporadic endocrine tumors, LOH 11q13 is identified in 5-50% of cases [7]. For example, biallelic somatic mutations in the *MEN1* gene in sporadic parathyroid adenomas were detected in 12–35% of cases [8]. Somatic mutations in the *MEN1* gene in sporadic pancreatic tumors can be found in about 25–44% [9, 10]. The percentage of somatic mutations of the *MEN1* gene in sporadic pituitary adenomas is low and is approximately 2–5% [11, 12], in adrenal tumors — less than 5% [13].

In 10–30% of familial cases and 60–80% of sporadic cases of MEN-1, there are no mutations in the *MEN1* gene identified, which may be due to large deletions of this gene, mutations in the promoter region, or other untranslated regions that are usually not analyzed in «routine» genetic testing [5, 14]. CpG island hypermethylation in the promoter regions of tumor suppressor genes is known to lead to loss of function of these genes [15]. Thus, hypermethylation of the promoter region of the *MEN1* gene was observed in the tissues of parathyroid adenomas at the 24–31 sites of CpG islands in patients with MEN-1, and the severity of clinical manifestation depended on the methylation rate [16]. Besides, the development of MEN-1-associated tumors in such patients may be due to other reasons: mutations in other genes that have not been discovered yet, epigenetic changes, and probably, a random combination of several tumors in one patient [1, 17, 18]. Detailed information about the MEN-1 syndrome phenocopies can be found in our review [19].

Menin protein and its functions

The menin protein consists of 610 amino acid residues whose sequence is not homologous to any known protein. Menin is expressed in all organs and tissues, but its expression varies depending on the type of tissue [20]. At the cellular level, it is mainly found in the nucleus, and a small amount can also be found in the cytoplasm and cell membrane. Menin is subject to post-translational modifications, such as phosphorylation (amino acid residues Ser394, Thr397, Thr399, Ser487, Ser543, Ser583), sumoylation, and palmitation. There are two main nuclear localization signals in the menin structure (NLS) — NLS1 (amino acid residues 479–497) and NLS2 (amino acid residues 588–608), plus the third additional NLS (NLSa, amino acid residues 546–572) [21]. When germinal and somatic mutations in the *MEN1* gene result in a shortened protein (nonsense mutations and frameshift mutations) and the loss of one or both main NLS, protein inactivation occurs. In missense mutations, proteasomes cause degradation of the synthesized protein preventing its functional activity. Menin does not have enzyme activity [21].

Studies have shown that menin interacts with many proteins (more than 50) in various protein complexes. Generally, menin-interacting proteins can be classified into four large groups: 1) transcription activators; 2) transcription repressors; 3) cell signaling proteins; and 4) other proteins with different functions (for example, regulation of DNA repair and the cell cycle, structural support, etc.) [20, 22]. Menin-interacting proteins are listed in Table 1.

Menin regulates multiple signaling pathways (Table 2). Besides, menin is regulated by various proteins and signaling pathways, including the ones that it itself regulates (Table 3). Interacting with different protein complexes, menin can participate in epigenetic regulation [37, 38].

The mechanisms by which the *MEN1* gene mutations (leading to the synthesis of the defective menin protein) cause the development of specific tumors, remain unclear.

NON-CODING RNAS IN MEN1

Non-coding RNAs (ncRNAs) do not have open reading frames and therefore, as their name implies, do not encode proteins. MicroRNA (miR) are short ncRNAs and consist of 20–24 base pairs. MicroRNAs repress gene expression with two mechanisms: complementary DNA binding in chromatin leading to RNA-induced suppression of gene transcription, or complementary binding of messenger RNA (mRNA) leading to its degradation and translation blocking [38]. microRNA encoding genes make up 1–5% of the human genome and control the expression of thousands of mRNAs, and several microRNAs can participate in the expression of a single mRNA [39]. Long ncRNAs (lncRNAs) are transcripts of about 200 or more base pairs that can interact with DNA and proteins, thereby participating in epigenetic regulation [40].

Table 1. Menin-interacting proteins [20, 21].

Transcription activators and repressors	c-MYB, MLL1, PEM, RUNX2, DAXX, HDACs mSIN3A, LEDGF, PRMT5, SuV39H1, DNMT1, FBP1, FOXA2, HLXB9/MNX1, JUND, c-MYC; NFkB – p50, p52, p65; nuclear receptors (AR, ERα, LXRα, PPARα, PPARγ, RXR, VDR); SMADs (SMAD1, SMAD3, SMAD5), SIRT1, SON, TCF3, TCF4, β-catenin; RNA-Pol-II (pSer5, pSer2) isoforms; SKIP
Signaling pathway proteins	AKT1, FOXO1, NM23β, GRB2, RAS, SOS1
Other proteins	RPA2, ASK, CHES1, FANCD2, GFAP, Vimentin, NMMHC-IIA, IQGAP1, ARS2, CHIP, HSP70

Table 2. Menin-regulated signaling pathways.

Signaling pathway	Menin effect	Sources
TGFβ (transforming growth factor beta)	↑	[23]
BMP (bone morphogenetic protein)	↑↓	[24]
Wnt	↑	[25]
Nuclear receptor	↑	[26, 27, 28]
Ras (small G proteins)	↓	[29, 30]
PI3K/Akt (protein kinase B) and FOXO	↓	[31, 32]
Hedgehog	↓	[33]

Note: ↓ — repression; ↑ — activation.

Table 3. Menin-regulating proteins and signaling pathways.

Signaling pathway	Effect on menin expression	Sources
Prolactin and its signaling pathways	↓	[34]
TGFβ (transforming growth factor beta)	↑	[24]
Somatostatin signaling pathway	↑	[35]
PI3K/Akt signaling pathway	↓	[36]
K-Ras-induced DNA methylation	↓	[29]

Note: ↓ — repression; ↑ — activation.

The change in microRNA expression is considered important in tumor initiation and progression, and there is already extensive data indicating its pathogenetic significance. For example, the literature describes differences in microRNA expression between normal tissues, benign and malignant pituitary tumors [41], parathyroid tumors [42], and adrenocortical tumors [43].

Menin mRNA is affected by various microRNAs. Besides, there is evidence that menin is involved in microRNA synthesis as a transcription factor (see Menin and microRNA below). Thus, changes in the epigenetic regulation of signaling pathways in the MEN-1 syndrome via microRNA may promote tumor growth.

Menin and microRNA

It has long been suggested that microRNA expression can be controlled by a transcription factor/factors or other microRNAs forming negative feedback loops, or that microRNAs together with transcription factors regulate the expression of target genes forming positive feedback loops [44]. Luzi et al. in their study have demonstrated that miR-24-1 directly binds to the 3'-untranslated region (3'-UTR) of menin mRNA and suppresses its expression [45]. They have also demonstrated that miR-24-1 is expressed only in the LOH-negative parathyroid adenomas in patients with genetically confirmed MEN-1 syndrome (with an intact wild-type allele) and is not expressed in the LOH-positive adenomas suggesting that menin is essential for the expression of this microRNA. Despite the residual expression of mRNA in the LOH-negative parathyroid adenomas in patients with MEN-1 (due to the intact wild-type allele), compared to the LOH-positive adenomas with no expression of the *MEN1* gene mRNA, there was no expression of menin itself in both subtypes indicating that the miR-24-1 overexpression has a negative effect on the menin mRNA. Thus, the authors have suggested a negative feedback loop with menin being essential for the expression of miR-24-1, while the latter suppresses menin expression in the absence of the LOH of the second allele of the *MEN1* gene, that is, «silences» this allele, which is consistent with Knudson's hypothesis [45]. Further on, Vijayaraghavan et al. have established that miR-24 directly reduces menin expression in the MIN6 cell lines (mouse insulinoma cell lines) and βlox5 (immortalized human β cell lines), and also have confirmed a negative feedback loop between menin and miR-24 [46]. Besides this study has demonstrated that miR-24-induced decrease in menin expression leads to decreased expression of the cell cycle inhibitors $p27^{kip1}$ и $p18^{ink4c}$ [46]. Ehrlich et al. in their study have demonstrated the increased expression of miR-24 in human cholangiocarcinoma cell lines as well as suppression of menin expression by this microRNA [47]. In another study, Luzi et al. have shown that menin binds directly to the primary RNA sequence of the precursor of this microRNA (pri-miR-24-1) and increases the expression of miR-24-1 [48].

In addition to miR-24, some other microRNAs were found that could suppress the expression of menin in different tissues. Yet another Luzi et al. study has demonstrated

that by interacting with the promoter of the miR-26a gene, menin induces the expression of this microRNA. The «silencing» of menin mRNA leads to decreased expression of miR-26a [49]. It is known that miR-26a is a regulator of SMAD1 protein, which plays a critical part in the cell cycle and growth [49]. Li et al. in their study have found that miR-421 expression in neuroblastoma tissues is increased compared to healthy tissues, which contributes to the proliferation, migration, and invasion of its cells [50]. In the neuroblastoma cell lines SHSY5Y, SHEP, and IMR-32, menin has shown to be a target of miR-421, which suppresses its expression by binding to the 3'-UTR of its mRNA. Whereas in the human neuroblastoma cell line SHSY5Y an increase in menin concentration leveled out the effects of miR-421 overexpression [50]. Lu et al. in their study on the MIN6 cell line have found that miR-17, whose expression is increased by high glucose levels in pancreatic β-cells, directly suppresses the expression of menin by binding to its 3'-UTR mRNA, thereby promoting the proliferation of pancreatic β-cells [51]. Hou et al. in their study have found a negative correlation between miR-762 and menin in the tissues of ovarian cancer. According to the data, miR-762 can directly suppress menin expression by binding to its 3'-UTR mRNA and activating the Wnt/β-catenin signaling pathway and thereby can boost the proliferation and metastasis of ovarian cancer cells [52]. A study by Gurung et al. has demonstrated that menin interacts with the ARS2 protein, a component of the nuclear CAP-binding complex that is crucial for the synthesis of certain microRNAs, and increases the processing of pri-let-7a and pri-miR-155 in prelet-7a and pre-miR-155, respectively, having no effect on the level of the precursors themselves [53]. The target of let-7a microRNA is the IRS2 protein, which plays an essential part in insulin signaling and insulin-induced pancreatic cell proliferation. These results show how menin suppresses cell proliferation, at least partially, by stimulating the processing of let-7a microRNA [53]. Ouyang et al. have demonstrated that miR-29 inhibits menin expression in the rat intestinal epithelial cell line [54]. Data on the mutual effect of menin and different microRNAs are presented in Table 4.

There are few studies on the evaluation of microRNA expression in tumors with the MEN-1 syndrome. Using microarrays, Luzi et al. have compared the expression of microRNA in seven parathyroid adenomas in patients with genetically confirmed MEN-1 syndrome (four adenomas have demonstrated LOH at locus 11q13, with the intact wild-type allele found in three adenomas) with two sporadic parathyroid adenomas (without any somatic mutations in *MEN1*) and two samples of healthy parathyroid tissues (fresh frozen material) [55]. The study has demonstrated that the expression of eight microRNAs (hsa-miR-4258, hsa-miR-664, hsa-miR-299-5p, hsa-miR-625, hsa-miR-877-5p, hsa-miR-3614-5p, hsa-miR-23c, hsa-miR-3938) differs between the LOH-negative parathyroid adenomas and the control, as well as the expression of two microRNAs (hsa-miR-1301, hsa-miR-664) differs between the LOH-positive parathyroid adenomas and the control. The expression of six microRNAs (hsa-miR-4258, hsa-miR-1301, hsa-miR-485-5p, hsa-miR-3944, hsa-miR-135b, hsa-miR-1261) differs between the LOH-positive and LOH-negative parathyroid adenomas. Meanwhile differences in expression of three microRNAs (miR-4258, miR-664 и miR-1301) found in the LOH-positive and LOH-negative parathyroid adenomas in patients with MEN-1are noteworthy. Thus, the expression of miR-4258 is suppressed in the LOH-positive parathyroid adenomas compared to the LOH-negative parathyroid adenomas, demonstrating that at least one wild-type allele is required for the expression of this microRNA. The expression of miR-4258 is higher in the LOH-negative parathyroid adenomas compared to the control. The expression of miR-664 is higher in the LOH-negative adenomas and is lower in the LOH-positive adenomas compared to the control. The expression of miR-1301 is higher in the LOH-positive parathyroid adenomas compared to the LOH-negative parathyroid and the control. Thus, the authors have concluded that the expression of some microRNAs requires at least one wild-type allele encoding the menin protein and that miR-4258, miR-1301, and miR-664 are the best prognostic and diagnostic markers to distinguish MEN-1-associated parathyroid adenomas, sporadic parathyroid adenomas, and healthy parathyroid tissues, as well as to distinguish MEN-1-associated parathyroid adenomas with or without LOH at locus 11q13 [55]. In the same study, the authors have searched for possible target genes known in the parathyroid tumor pathogenesis for the identified microRNAs with altered expression using the ComiR tool computer algorithm. MiR-4258, in particular, suppresses the expression of the *CCND1* gene encoding cyclin D1 (a positive regulator of cell cycle progression). Thus, a decrease in miR-4258 expression following the loss of the wild-type allele of the *MEN1* gene may be responsible for the induction of uncontrolled parathyroid cell growth. Increased expression of miR-1301 with the loss of the wild-type allele of the *MEN1* gene suppresses the expression of the *CDKN1B*, *RB1*, *CTNNB1*, and *RET* genes. MiR-664 suppresses the expression of the *CDKN2C* gene and the parafibromin-encoding tumor suppressor gene *CDC73* [55].

Grolmusz et al. have compared 16 parathyroid gland lesions in patients with genetically confirmed MEN-1 syndrome and 40 sporadic parathyroid gland lesions [56]. The authors have analyzed the potential presence of an intact wild-type allele of the *MEN1* gene with an immunohistochemical (IHC) test. The results have not shown nuclear menin staining in all MEN-1-associated parathyroid lesions and in 28% (11/40) of sporadic parathyroid lesions.

The study of somatic mutations in the tissues of sporadic parathyroid lesions of the glands has revealed mutations in the *MEN1* gene in 25% of cases (10/40). Thus, the authors have calculated the sensitivity (86%) and specificity (87%) of the IHC method for the identification of somatic mutations. The expression of microRNAs (hsa-miR-24, hsa-miR-28, hsa-miR-326, hsa-miR-484, hsa-miR-637, hsa-miR-744) was analyzed using the material from paraffin-embedded blocks by real-time quantitative PCR. The expression of hsa-miR-637 was not identified in all samples. There were no significant differences in the remaining microRNAs between the menin-positive and menin-negative tissues of parathyroid lesions, regardless of the presence or absence of a germinal mutation in the *MEN1* gene. However, the expression of hsa-miR-24 and hsa-miR-28 was higher in sporadic parathyroid lesions compared to MEN-1-associated lesions. Besides, when the group of sporadic parathyroid lesions was further divided into menin-positive and menin-negative ones, the expression of these microRNAs was higher in both groups than in MEN-1-associated lesions [56].

Table 4. Mutual effect of menin and microRNAs.

Menin-affecting MicroRNAs			
MicroRNAs	**Effect**	**Interaction**	**Biological research sample, source reference**
miR-24-1§	↓ expression	Binds to the 3'UTR of menin mRNA	BON1 [45]
miR-24§	↓ expression	Binds to the 3'UTR of menin mRNA	MIN6, βlox5 [46]
miR-24	↓ expression	Correlation of expression levels	Mz-ChA-1, TFK-1, SG231, CCLP-1, HuCC-T1, HuH-28 [47]
miR-421	↓ expression	Binds to the 3'UTR of menin mRNA	SHSY5Y, SHEP, and IMR-32 [50]
miR-17	↓ expression	Binds to the 3'UTR of menin mRNA	MIN6 [51]
miR-762	↓ expression	Binds to the 3'UTR of menin mRNA	SKOV3 [52]
miR-29b	↓ expression	Binds to a single site of the coding sequence of menin mRNA	IECs [54]
Menin-affected MicroRNAs			
MicroRNAs	**Effect**	**Interaction**	**Biological research sample, source reference**
miR-24§	↑ expression	Increased expression due to menin overexpression	MIN6, βlox5 [46]
miR-24-1§	↑ expression	Menin binds to the RNA of the precursor of this microRNA, pri-miR-24-1	BON1 [48]
miR-26a	↑ expression	Decreased expression of this microRNA caused by the «silencing» of menin mRNA. Menin binds to the promoter of the miR-26a gene and induces its expression	hADSCs [49]
let-7a*	↑ pri-miR processing into pre-miR, increasing the level of mature microRNA. Has no effect on the pri-miR level	Let-7a and miR-155 levels were reduced by the excision of the *Men1* gene in the cell line	MEFs [53]
miR-155*			

Cell lines listed: BON1 – human pancreatic neuroendocrine tumor cell line; MIN6 – mouse insulinoma cell line; βlox5 – immortalized human pancreatic β cells; Mz-ChA-1, TFK-1, SG231, CCLP-1, HuCC-T1, HuH-28 – human cholangiocarcinoma cell lines; SHSY5Y, SHEP, and IMR-32 – neuroblastoma cell lines; SKOV3 – human ovarian adenocarcinoma cell line; IECs – rat intestinal epithelial cells; hADSCs – human stem cells isolated from adipose tissue (in this case, induced into osteoblasts by differentiation); MEFs – mouse embryonic fibroblasts.
↓ – decrease, ↑ – increase.
§ – miR-24 transcription from chromosome 9 (miR-24-1) and chromosome 19 (miR-24-2), both miR-24 (miR-24-1 and miR-24-2) are identical in structure and differ only in the chromosome of origin [46].
* – affects the level of mature microRNA, but not the level of its precursor (see in the text).

Lines et al. in their study have analyzed the expression of miR-15a, miR-16-1 and let-7a in miR-15a, miR-16-1 and let-7a in pituitary adenomas in mice with heterozygous knockout of the *Men1* gene [57]. The expression of all three microRNAs was significantly suppressed compared to the control group (healthy mouse pituitary glands). The study has also demonstrated a significantly increased expression of *Ccnd1* gene mRNA and cyclin D1 protein in pituitary adenomas of *Men1+/-* mice compared to healthy mouse pituitary glands. An inverse correlation was found between the levels of miR-15a and miR-16-1 and *Ccnd1* mRNA, indicating a potential regulation of cyclin D1 by these microRNAs. This opinion was confirmed in cell cultures when the introduced antagonists to miR-15a and miR-16-1 led to a significant increase in the expression of cyclin D1 [57]. The analysis of mRNA expression of the let-7a microRNA target – *Kras* – revealed a significant increase in *Kras* expression in pituitary adenomas of *Men1 +/-* mice, without a significant inverse correlation between *Kras* and let-7a expression. The authors of this study have also found that the lack of menin expression in cell culture leads to decreased expression of miR-15a, but not of miR-16-1. Besides, miR-15a and miR-16-1 do not

directly affect the expression of menin, indicating the absence of feedback loops [57].

Today, the search for microRNAs affecting menin and its functions continues. Nagy et al. in their study have analyzed the literature and compared data on significantly different microRNA expression in healthy tissues and the pituitary, parathyroid, and adrenocortical tumors (benign and malignant), and noticed changes in the expression of microRNAs caused by DLK1-MEG3 — one of the largest microRNA clusters in the human genome. MicroRNAs from this cluster regulate signaling pathways that are often involved in tumor genesis, i.e., mTOR, MAPK, Wnt/β-catenin, p53, where the menin protein is also involved. However, there are no experimental data on the potential association between the DLK1-MEG3 cluster microRNAs and *MEN1* [58]. Nagy et al. in their article have suggested another potential association between *MEN1* and microRNA via the miR-142-3p microRNA gene, which is regulated by the menin protein in human osteosarcoma tissues [58]. The adrenocorticotropic hormone is known to induce the expression of miR-142-3p microRNA, which in turn affects glucocorticoid receptors in the adrenal glands. There are also data showing an increased expression of the glucocorticoid receptor alpha in cortisol-producing adrenocortical adenomas compared to hormone-inactive adenomas and healthy tissues [59]. Thus, a hypothesis has been suggested about regulatory loops promoting oncogenesis in the adrenal tissues in the absence of the menin protein: a decrease in the expression of menin in adrenocortical tumors would lead to a decrease in the production of miR-142-3p microRNA and thereby to an increase in the glucocorticoid receptors, causing tumor growth. To confirm this potential pathway, the authors have proposed to conduct studies demonstrating the regulation of miR-142-3p expression by menin in the adrenocortical tissues [58].

Menin and lncRNAs

Some lncRNAs are known to be found in chromatin-remodeling protein complexes that can suppress gene expression [60, 61]. There are only scarce data in the literature on the interaction of menin and lncRNAs. So, Modali et al. in their study have described the epigenetic regulation of Meg3 lncRNA by menin in pancreatic β-cells and identified the c-Met proto-oncogene (hepatocyte growth factor receptor) as a target gene for Meg3 [62]. Using a mouse insulinoma cell line (MIN6 cells), menin was found to activate the Meg3 lncRNA causing its increased expression by histone H3 lysine 4 trimethylation and CpG hypomethylation at the CRE site of the *Meg3* gene promoter. Such an effect did not occur in the absence of the menin protein. Increased expression of the Meg3 lncRNA caused a decrease in the expression of the c-Met proto-oncogene leading to suppressed tumor cell activity in MIN6. Comparison of the pancreatic cells in wild-type mice and *Men1* +/- mice has proven the same (a significant decrease in the expression of Meg3 lncRNA in tumor cells in *Men1* +/- mice and, therefore, increased c-Met staining compared to normal β-cells in the same pancreatic tissue slice). The regulation of MEG3 and c-MET was further evaluated in frozen samples of pancreatic β-cell tumors from patients with and without *MEN1* mutations: four of the five samples with *MEN1* mutations and all three mutation-free samples demonstrated a significant decrease in the expression of MEG3 lncRNA, whereas all five pancreatic tumors with mutations and two of the three mutation-free pancreatic tumors showed a significant increase in the expression of the c-MET protein. It is interesting that the expression of MEG3 and c-MET changed in sporadic human insulinomas with hypermethylation at the CRE site of the *MEG3* promoter, as well as in patients with *MEN1* mutations [62].

CONCLUSION

Therefore, recent studies have revealed the mechanisms of tumor growth with the *MEN1* gene inactivation due to its mutations, as well as the likely epigenetic mechanisms of the *MEN1* gene «silencing», which can explain both the growth of MEN-1-associated tumors and the MEN-1 syndrome phenocopies. So, menin was found to regulate the expression of several ncRNAs, which in turn regulate the transcription of genes encoding growth factors that affect cell proliferation. Thus, this may be one of the mechanisms of tumor growth in the *MEN1* gene mutations. Besides, several ncRNAs that regulate the expression of menin and their abnormal expression may explain menin inactivation without the identified mutation in the *MEN1* gene. Of course, we should not rule out the possibility of undetected mutations in other genes, or a chance of a random combination of several tumors within the MEN-1 syndrome phenocopies. The identification of any menin-interacting agents significantly increases the knowledge of its physiopathology and tumor growth within the MEN-1 syndrome. Menin is involved in epigenetic processes, and its inactivation may lead to epigenetic changes promoting tumor growth. Given that epigenetic changes can be reversible, it is possible to return the epigenome to its original (normal) state by tackling with them. Therefore, nowadays, the development of targeted drugs is being proposed for the correction of epigenetic changes. Studies of the complex networks of the menin molecular pathway will contribute to the development of new therapeutic methods for the treatment of MEN-1 syndrome.

ADDITIONAL INFORMATION

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