The involvement of microRNAs in Multiple Endocrine Neoplasia type 1

Cinzia Aurilia, Teresa Iantomasi

Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

ABSTRACT

Multiple endocrine neoplasia type 1 (MEN1) syndrome is caused by mutations in the MEN1 gene, resulting in reduced or completely absent production of the oncosuppressor menin. This genotype often results in the occurrence of tumors in endocrine tissues (parathyroids, pituitary gland, and endocrine pancreas) and beyond. However, although more than 1,500 MEN1 mutations have been identified, no genotype-phenotype relationship has been observed in this syndrome, suggesting that specific clinical phenotypes may be due to the action of other factors, such as epigenetics. Over the past 20 years, it has been seen that deregulation of microRNA (miRNA) expression may play a key role in the onset and progression of several diseases, including MEN1. Moreover, recently, in addition to their intracellular counterparts, a new class of extracellular, or circulating, miRNAs has been identified whose variation in expression levels seems to be associated with specific diseases, including cancers. In this review, we look at the miRNAs that might be involved in the pathogenesis of MEN1, and therefore represent possible targets for developing new therapies for the syndrome. In addition, we discuss the possibility of using some circulating miRNAs as potential future diagnostic and prognostic biomarkers of MEN1.

KEYWORDS

MEN1, parathyroids, pituitary, pancreas, miRNAs, tumor.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a hereditary tumor syndrome caused by mutations in the *MEN1* gene, culminating in reduced or completely absent production of the oncosuppressor protein menin. The main clinical feature of MEN1 is the onset of more than two tumors in endocrine glands (i.e., pituitary gland, parathyroids, pancreas) in a single patient [1]. Although these are the main organs involved in MEN1, twenty different tumors have been associated with the syndrome, which can also develop in non-endocrine tissues [2].

MEN1 is a rare disease with an estimated prevalence of 1 in 30000 individuals; furthermore, no gender or ethnic predilection has been observed. The syndrome is inherited in an autosomal dominant fashion, so the probability of transmission of the disease to offspring is 50% [2].

Today, MEN1 is diagnosed by means of a genetic test that searches for the presence of mutations in the gene; alternatively, the diagnosis can be made on a clinical basis if the patient has two of the three main tumors associated with the syndrome, or if the patient has a tumor in one of the glands mentioned above and also has a first-degree relative affected by MEN1[3].

Rather than treatment of MEN1-associated tumors, it would be more correct to speak of management of the syndrome, since the administration of pharmacological therapies often tends to keep the over-secretion of hormones under control, while surgery is not always curative, given the high prevalence of recurrences observed in patients [4].

All this must motivate the scientific community to seek new possible therapies able to resolve the condition or even prevent the onset of tumors in MEN1 patients.

Article history

Received 25 Nov 2024 – Accepted 17 Dec 2024

Contact

Teresa Iantomasi; teresa.iantomasi@unifi.it

Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

A very distinctive characteristic of MEN1 syndrome is the total lack of genotype-phenotype correlation. In fact, individuals from the same family carrying identical mutations tend to develop different tumors, suggesting that other molecular mechanisms, including epigenetics, may be involved in the pathogenesis of the syndrome [5].

Epigenetics includes all those molecular mechanisms that affect gene expression without altering the DNA nucleotide sequence, such as histone modifications, DNA methylations, and the action of non-coding RNAs (ncRNAs) [1].

In recent years, epigenetics has attracted considerable attention among researchers as a possible molecular target for the treatment of several diseases, including MEN1 [6]. In particular, it has been observed that DNA methylations and histone modifications may contribute to the progression of MEN1-associated pancreatic neuroendocrine tumors [2]. Furthermore, a key role in the onset and progression of the syndrome seems to be played by microRNAs (miRNAs). These small molecules are ncRNAs capable of negatively regulating gene expression by interacting with the 3'-UTR of the mRNA target, inhibiting its translation and protein production. Since altered expression of

one or more miRNAs could be the basis of the onset and progression of various diseases, including cancer, they may be a valid therapeutic target [7-9].

Recently, in addition to the intracellular localization, these molecules have also been identified in several biological fluids (urine, plasma, serum, etc.), where they are referred to as circulating miRNAs (c-miRNAs) and could represent an interesting means of intercellular communication [10-14]. Over the years, it has emerged that changes in the expression profiles of c-miRNAs could provide clues about an individual's pathological or physiological states, making them good candidates as non-invasive biomarkers for use in clinical practice [15-19].

Here, we review the miRNAs that could be involved in MEN1 pathogenesis, and which might therefore be considered possible targets for the development of novel molecular therapies for the syndrome. Moreover, we also discuss the possibility of using c-miRNAs as potential non-invasive diagnostic and prognostic biomarkers in MEN1 patients.

miRNAs in MEN1 tumors

The literature contains few papers focusing on the specific involvement of miRNAs in MEN1-associated tumors. Indeed, perhaps due to the rarity of the disease and the consequent scarcity of available MEN1 tissues, or perhaps because of the lack of suitable cellular and animal models, most studies on the deregulation of miRNA expression have been conducted in non-MEN1 neuroendocrine tumors.

The first study of this specific topic was carried out by Luzi *et al*.[20] in 2012. They demonstrated that in MEN1 parathyroid adenomas (PTAs), under heterozygous (non-LOH) conditions, miR-24-1 was able to bind the 3'-UTR of *MEN1* mRNA and prevent menin production. They suggested that this mechanism mimics "Knudson's second hit" and induces tumorigenesis of the parathyroid glands before the loss of heterozygosity (LOH) occurs.

A few years later, the same research group demonstrated the presence of an autoregulatory negative feedback loop between miR-24-1, *MEN1* mRNA, and menin, which could play a key role in the occurrence of MEN1-associated tumors [21].

Subsequently, Luzi *et al*.[22] compared the expression profile of 1890 miRNAs in LOH PTAs, non-LOH PTAs, and sporadic PTAs, showing that three miRNAs (miR-664, miR-1301, and miR-4258) were differentially expressed between the tissues under investigation. Furthermore, *in silico* analyses showed that these miRNAs could target genes involved in familial forms of parathyroid tumors, reinforcing the hypothesis that they are also involved in the development of MEN1 PTAs.

In the same year, Grolmusz *et al*.[23] compared the expression of six miRNAs (miR-744, miR-24, miR-637, miR-28, miR-326, and miR-484) in sporadic and MEN1-associated hyperparathyroidism tissue samples. They found that miR-24 and miR-28 had higher expression in sporadic hyperparathyroidism tissues than in MEN1 tissues, which led them to think that these miRNAs might be involved in parathyroid tumorigenesis regardless of the presence of mutations in the *MEN1* gene.

Another interesting study of the involvement of miRNAs in the development of MEN1-associated PTAs was carried out by Hwang *et al*.[24]. They first performed a microarray analysis of 887 miRNAs in sporadic PTAs, MEN1 PTAs, and healthy parathyroids, and then, by real-time (RT) qPCR validation, showed that only miR-199b-5p was differentially expressed between sporadic and hereditary tumors compared with normal tissue. In addition, the altered expression of this miRNA was also negatively associated with parathyroid hormone (PTH) production in sporadic tumors, whereas this association was not observed in MEN1 tumors. Finally, ROC curve analysis showed that this miRNA could also represent a good diagnostic biomarker in discriminating sporadic from hereditary parathyroid tumors with a sensitivity of 67% and a specificity of 100%.

As regards the study of miRNAs in MEN1 gastro-entero-pancreatic tumors, Lu *et al*. [25], in 2015, observed that murine insulinoma cells showed increased expression of miR-17 after being treated with high doses of glucose. Subsequent *in silico* and *in vitro* analyses revealed that this miRNA was able to inhibit the expression of menin, suggesting that increased cell growth after glucose stimulation was due to the suppression of the oncosuppressor by miR-17.

Recently, Luzi *et al*. [26], using Next Generation Sequencing (NGS) to compare the expression profile of different miRNAs in various tissue types (pancreatic neuroendocrine tumor, gastrinoma, and healthy pancreas tissue), found that several miRNAs were differently deregulated between tissues. Subsequent RT-qPCR validation showed that miR-1468-5p, miR-625-5p, miR-625-3p, and miR-215-5p were upregulated and miR-1301-3p and miR-212 downregulated in both tumor types compared with control tissue. In addition, the use of gene set enrichment analysis showed these miRNAs to be organized in a network of interactions both with genes involved in chromatin remodeling and pancreatic cancer development (i.e., *MEN1*, *DAXX*, and *ATRX*) and with classic oncosuppressors that are generally found to be mutated in neuroendocrine carcinomas (i.e., P53 and Rb).

The hypothesis that miR-24 plays a key role in the pathogenesis of MEN1 was corroborated by Vijayaraghavan *et al*. [27], who confirmed the existence of a negative feedback mechanism between miR-24, *MEN1* mRNA, and menin in endocrine pancreas cell lines. These authors found that miR-24 was able to negatively regulate the expression of menin by targeting *MEN1* mRNA, and that menin could, in turn, regulate miR-24 expression by binding in the upstream region of this miRNA. Furthermore, they observed that miR-24 overexpression and consequent inhibition of menin resulted in altered expression of two major cell cycle inhibitors (p27kip1 and p18ink4c), inducing an increase in cell proliferation. As this occurred mainly in cells whose growth was not very high, the authors hypothesized that miR-24 may be responsible for increased beta-cell proliferation and pancreatic islet hyperplasia in the early stage of MEN1 tumorigenesis.

The only work on the action of miRNAs in MEN1-associated pituitary adenoma was performed by Lines *et al*. [28], who observed not only a downregulation of miR-15a and miR-16-1 in pituitary tumors from *MEN1*$^{+/-}$ mice compared with healthy tissue, but also an inverse correlation with cyclin D1 expression. In addition, further studies in the AtT20 cell model showed that *MEN1* gene deletion led to a decrease in miR-15a

expression, suggesting that there may be a link between these two molecules and that this mechanism may contribute to the development of pituitary tumors in MEN1 patients.

c-miRNAs in MEN1

The discovery of a specific c-miRNA expression profile able to discriminate MEN1 patients from healthy individuals, and also be unequivocally linked to specific tumors, could pave the way for the development of new non-invasive diagnostic and prognostic strategies to complement those currently used to allow timely diagnosis and treatment of the disease.

To date, only two studies have focused on the search for c-miRNAs in MEN1.

Kooblall *et al.* [29], comparing the expression profile of miRNAs in the serum of MEN1 patients and healthy controls, identified four deregulated miRNAs (miR-125a-3p, miR-582-3p, miR-3156-5p, and miR-3168) in patients vs. controls. Subsequent qPCR validation confirmed that miR-3156-5p was significantly downregulated in the serum of the MEN1 vs. the healthy population. Moreover, subsequent *in vitro* analysis showed that the reduction of menin expression correlated with the downregulation of miR-3156-5p expression. The authors finally found that the miRNA in question was capable of targeting and negatively modulating the expression of mortality factor 4-like 2 (MOR4FL2), which, however, did not seem to affect cell viability, migration, or apoptosis.

The second study was conducted by Trukhina *et al.* [30], who analyzed the expression of c-miRNAs in the plasma of MEN1 patients with a confirmed genetic diagnosis (g-MEN1), in patients presenting the syndrome phenocopy condition (p-MEN1), and in healthy controls. They demonstrated differences in expression of miRNAs between g-MEN1 and p-MEN1 (i.e., miR-25-5p, miR-30a-3p, miR-32-5p, miR-141-3p, miR-215-5p, miR-335-5p, miR-425-3p miR-501-3p, miR-576-5p, miR-760 and miR-3613-5p), between g-MEN1 and controls (i.e., miR-375, miR-144-5p, miR-532-3p and miR-1976), and between p-MEN1 and controls (i.e., miR-98-5p, miR-191-5p and miR-944). Exploration of c-miRNAs in MEN1 is still in its infancy and further studies are needed to define a future use of these molecules in clinical practice.

Table I lists the main miRNAs possibly involved in MEN1 syndrome, summarizing their targets and possible effects.

Conclusions

The above studies seem to suggest that the complex network established between the various miRNAs, *MEN1* mRNA, and menin could be a focal point in the development and progression of MEN1 tumorigenesis, and possibly explains the absence of genotype-phenotype correlation in the pathology.

In particular, miR-24 appears to play a key role in the pathogenesis of the syndrome, representing an intermediate epigenetic step before menin expression is completely lost and neoplasia arises. An interesting aspect of this miRNA is that its inhibitory action against menin has also been found in tumors

Table I Summary of miRNAs possibly involved in MEN1 syndrome.

CELLS/TISSUE	miRNAs	POTENTIAL ROLE IN MEN1	REFERENCE
Parathyroid adenomas	miR-24-1	Targeting *MEN1* mRNA and promoting parathyroid tumorigenesis	20, 21
Parathyroid adenomas	miR-664, miR-1301, and miR-4258	Parathyroid tumorigenesis, possibly regulating the expression of genes involved in familial forms of parathyroid tumors	2
Parathyroid adenomas	miR-24 and miR-28	Parathyroid tumorigenesis	23
Parathyroid adenomas	miR-199b-5p	Parathyroid tumorigenesis and PTH production	24
Murine insulinoma cells	miR-17	Promoting cell growth, by targeting *MEN1* mRNA	25
Pancreatic neuroendocrine tumors	miR-1468-5p, miR-625-5p, miR-625-3p, miR-215-5p, miR-1301-3p and miR-212	Possible role in pancreatic tumorigenesis	26
Endocrine pancreas cell lines	miR-24-1	Increase in beta-cell proliferation and pancreatic islet hyperplasia development, by regulating *MEN1* mRNA expression	27
Murine pituitary tumors	miR-15a and miR-16-1	Possible role in pituitary tumor development, by regulating *CCND1* mRNA expression	28
Serum and BON1 cells	miR-3156-5p	Targeting *MOR4FL2* mRNA and possible diagnostic biomarker	29
Plasma	miR-25-5p, miR-30a-3p, miR-32-5p, miR-98-5p, miR-141-3p, miR-144-5p, miR-191-5p, miR-215-5p, miR-335-5p, miR-375, miR-425-3p miR-501-3p, miR-532-3p, miR-576-5p, miR-760, miR-944, miR-1976 and miR-3613-5p	Possible diagnostic biomarkers	30

not associated with the syndrome, such as lung and liver cancer [31,32], where menin nevertheless exerts oncosuppressor activity; this further supports the hypothesis that miR-24 could be an initiator of the tumorigenesis induced by menin loss. One possibility might therefore be to decrease the expression of miR-24, before loss of the second allele of the *MEN1* gene definitively occurs, through the use of various strategies (i.e., anti-miRNA oligonucleotides, miRNA inhibitors, miRNA sponges, and miRNA masks) [6]. This could lead to recovery of the menin protein and prevent the onset of tumors in the patient.

Another important point concerns c-miRNAs, which might be considered valid non-invasive diagnostic and prognostic biomarkers useful for assessing the patient's status over time. This would certainly improve surveillance of the disease, as well as the patient's quality of life, and also direct him or her toward a specific pharmacological treatment in a timely manner.

Undoubtedly, the rarity of the disease and low availability of suitable tissues and cellular models make it particularly difficult to study the pathogenetic mechanisms underlying the development and progression of MEN1.

However, it falls to the scientific community to identify the best scientific approach to further implement knowledge about the molecular mechanisms underlying the syndrome. Therefore, in this work, we reviewed the miRNAs that could be involved in the pathogenesis of MEN1 and that could represent possible targets for the development of innovative therapeutic strategies for the treatment of the several types of tumor associated with the syndrome.

References

1. Donati S, Ciuffi S, Marini F, et al. Multiple endocrine neoplasia type 1: the potential role of microRNAs in the management of the syndrome. Int J Mol Sci. 2020;21(20):7592.
2. Brandi ML, Agarwal SK, Perrier ND, Lines KE, Valk GD, Thakker RV. Multiple endocrine neoplasia type 1: latest insights. Endocr Rev. 2020;42(2):133-70.
3. Falchetti A. Genetic screening for multiple endocrine neoplasia syndrome type 1 (MEN-1): when and how. F1000 Med Rep. 2010;2:14.
4. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4). Mol Cell Endocrinol. 2014;386(1-2):2-15.
5. Feng Z, Ma J, Hua X. Epigenetic regulation by the menin pathway. Endocr Relat Cancer. 2017;24(10):T147-T159.
6. Marini F, Brandi ML. Role of miR-24 in multiple endocrine neoplasia type 1: a potential target for molecular therapy. Int J Mol Sci. 2021;22(14):7352.
7. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018;9:402.
8. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet. 2011;12(2):99-110.
9. Ipsaro JJ, Joshua-Tor L. From guide to target: molecular insights into eukaryotic RNA-interference machinery. Nat Struct Mol Biol. 2015;22(1):20-8.
10. Cogswell JP, Ward J, Taylor IA, et al. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. J Alzheimers Dis. 2008;14(1):27-41.
11. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PloS One. 2012;7(3):e30679.
12. Weber JA, Baxter DH, Zhang S, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010;56(11):1733-41.
13. Zhou Q, Li M, Wang X, et al. Immune-related microRNAs are abundant in breast milk exosomes. Int J Biol Sci. 2012;8(1):118-123.
14. da Silveira JC, Veeramachaneni DNR, Winger QA, Carnevale EM, Bouma GJ. Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible new form of cell communication within the ovarian follicle. Biol Reprod. 2012;86(3):71.
15. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513-8.
16. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18(10):997-1006.
17. Chim SS, Shing TK, Hung EC, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem. 2008;54(3):482-90.
18. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008;141(5):672-5.
19. Materozzi M, Merlotti D, Gennari L, Bianciardi S. The potential role of miRNAs as new biomarkers for osteoporosis. Int J Endocrinol. 2018;2018:2342860.
20. Luzi E, Marini F, Giusti F, Galli G, Cavalli L, Brandi ML. The negative feedback-loop between the oncomir Mir-24-1 and menin modulates the Men1 tumorigenesis by mimicking the "Knudson's second hit". PLoS One. 2012;7(6):e39767.
21. Luzi E, Marini F, Ciuffi S, Galli G, Brandi ML. An autoregulatory network between menin and pri-miR-24-1 is required for the processing of its specific modulator miR-24-1 in BON1 cells. Mol Biosyst. 2016;12(6):1922-8.
22. Luzi E, Ciuffi S, Marini F, Mavilia C, Galli G, Brandi ML. Analysis of differentially expressed microRNAs in MEN1 parathyroid adenomas. Am J Transl Res. 2017;9(4):1743-53.
23. Grolmusz VK, Borka K, Kövesdi A, et al. MEN1 mutations and potentially MEN1-targeting miRNAs are responsible for menin deficiency in sporadic and MEN1 syndrome-associated primary hyperparathyroidism. Virchows Arch Int J Pathol. 2017;471(3):401-11.
24. Hwang S, Jeong JJ, Kim SH, et al. Differential expression of miRNA199b-5p as a novel biomarker for sporadic and hereditary parathyroid tumors. Sci Rep. 2018;8(1):12016.
25. Lu Y, Fei XQ, Yang SF, Xu BK, Li YY. Glucose-induced microRNA-17 promotes pancreatic beta cell proliferation through down-regulation of menin. Eur Rev Med Pharmacol Sci. 2015;19(4):624-9.
26. Luzi E, Pandolfini L, Ciuffi S, et al. MicroRNAs regulatory networks governing the epigenetic landscape of MEN1 gastro-entero-pancreatic neuroendocrine tumor: a case report. Clin Transl Med. 2021;11(4):e351.
27. Vijayaraghavan J, Maggi EC, Crabtree JS. miR-24 regulates menin in the endocrine pancreas. Am J Physiol Endocrinol Metab. 2014;307(1):E84-92.
28. Lines KE, Newey PJ, Yates CJ, et al. MiR-15a/miR-16-1 expression inversely correlates with cyclin D1 levels in MEN1 pituitary NETs. J Endocrinol. 2018;240(1):41-50.
29. Kooblall KG, Stokes VJ, Shariq OA, et al. miR-3156-5p is downregulated in serum of MEN1 patients and regulates expression of MORF4L2. Endocr Relat Cancer. 2022;29(10):557-68.
30. Trukhina DA, Mamedova EO, Nikitin AG, Koshkin PA, Belaya ZE, Melnichenko GA. [Plasma miRNA expression in patients with genetically confirmed multiple endocrine neoplasia type 1 syndrome and its phenocopies]. Probl Endokrinol (Mosk). 2024;69(6):70-85.
31. Pan Y, Wang H, Ma D, et al. miR-24 may be a negative regulator of menin in lung cancer. Oncol Rep. 2018;39(5):2342-50.
32. Ehrlich L, Hall C, Venter J, et al. miR-24 inhibition increases menin expression and decreases cholangiocarcinoma proliferation. Am J Pathol. 2017;187(3):570-80.