EXPRESSION OF MENIN IN THE HUMAN THYROID GLAND

O.M. Capraru^{1,3}, M. Decaussin-Petrucci⁴, M.O. Joly⁵, A. Borda^{2,*}, I.S. Fanfaret², F. Borson-Chazot^{3,6}, S. Selmi-Ruby³

University of Medicine and Pharmacy Targu Mures, ¹Dept. of Physiology, ²Dept. of Histology, Targu Mures, Romania, ³Université Claude Bernard Lyon 1 - INSERM Unité 1052, Centre de recherche en cancérologie, ⁴Centre Hospitalier Lyon Sud, Pierre-Bénite, Hospices Civils de Lyon, Université Lyon 1 - Service d'Anatomie et Cytologie Pathologiques, ⁵Hôpital Edouard Herriot, Hospices Civils de Lyon, Université Lyon 1 - Service d'Anatomie et Cytologie Pathologiques, ⁶Groupement hospitalier Est, Bron, Hospices Civils de Lyon, Université Lyon 1 - Fédération d'endocrinologie, Lyon, France

Abstract

Introduction. The expression of menin in the thyroid gland has long been debated. Animal models with targeted inactivation of menin in the thyroid gland have shown that its inactivation might play a role in the progression to a more aggressive type of cancer. Human studies are conflicting, some have identified mutations in the MEN1 gene in a sub-type of oncocyctic thyroid carcinomas, while others have not identified a higher prevalence of thyroid cancer in MEN1 patients.

Objective. To analyze the immunohistochemical expression of menin in different types of thyroid carcinomas.

Materials and methods. 48 thyroid tumours (12 papillary thyroid carcinomas (PTC), 6 anaplastic thyroid carcinomas (ATC), 12 poorly differentiated thyroid carcinomas (PDTC), 5 medullary thyroid carcinomas (MTC), 5 oncocyctic follicular carcinomas (OC), 3 oncocyctic adenomas (OA) and 5 goiters (G)) were tested for nuclear expression of menin using an anti-menin antibody. The expression was considered positive, negative or decreased.

Results. The expression of menin was positive, identical to normal tissue, in 39 cases (81.25%). The expression was decreased (n=8) or absent (n=1) in 9 tumours (18.75% - 2 PTC, 5 PDTC, 2 OC) accounting for 42% (5/12) of the PDTC and 40% (2/5) of the OC.

Conclusions. Our results show that the expression of menin is generally preserved in human thyroid carcinomas, but it can be decreased or absent in certain types of thyroid cancer. Further molecular studies are needed to evaluate to potential of menin protein in tumorigenesis.

Key words: menin, thyroid carcinoma, immunohistochemistry.

INTRODUCTION

Multiple Endocrine Neoplasia type 1 (MEN1) or Wermer syndrome was first described in 1954

by Moldawer (1) and Wermer (2) as a hereditary syndrome with an autosomal dominant transmission with high penetrance. Almost 90% of the individuals carrying a mutation in the MEN1 gene will develop the disease, the first lesion as early as 8 years (3). The syndrome is caused by the inactivation of the MEN1 gene which encodes the protein called menin. Usually, patients inherit the germline mutation affecting one copy of the MEN1 gene, and, at some point, there is a somatic mutation in the remaining normal copy of the gene leading to tumorigenesis, a two hit hypothesis issued by Knudson (4).

The MEN1 syndrome is characterized by the development of hyperplasia and tumours in certain endocrine glands (parathyroids, pituitary, pancreas, gastro-intestinal system) (5). Most of the lesions in the MEN1 syndrome are usually benign but a long-standing untreated disease can lead to the development of malignant tumours (6).

As the thyroid is not among the affected glands of the MEN1 syndrome, studies do not systematically collect data regarding its pathology, thus leading to controversial data in the literature. Some reports found that thyroid pathology could also be present in MEN1 syndrome in a range of 2.6% – 25%, mostly represented by adenomas, colloid goiters, or more rarely, by carcinomas (5, 7). Most of these thyroid disorders have been discovered incidentally during neck ultrasound for hyperparathyroidism.

The aim of our study was to evaluate by immunohistochemistry the *in vivo* expression of the menin protein in a large series of thyroid tumours.

*Correspondence to: Angela Borda, Professor, MD, PhD, Department of Histology, University of Medicine and Pharmacy of Targu-Mures, 37 Gheorghe Marinescu Street 540139 Targu Mures, Romania, E-mail: aborda2001@yahoo.com

MATERIALS AND METHODS

Forty-eight cases from the archives of the “Service d’anatomie et de cytologie pathologiques, Centre de Biologie Sud, Centre Hospitalier Lyon Sud”, France were included in the study. All tissue samples used in this study were obtained from previous surgical resections. None of the patients included were known to have MEN1 syndrome at the time of the study. The study was performed after the approval of the local human studies committee.

All the cases were reviewed by the author and an endocrine pathologist on haematoxylin-eosin stained slides. According to the World Health Organization classification of thyroid tumours (8), the 48 cases included in the study consisted of 12 papillary thyroid carcinomas (PTC), 12 poorly differentiated thyroid carcinomas (PDTC), 6 anaplastic thyroid carcinomas (ATC), 5 medullary thyroid carcinomas (MTC), 5 oncocyctic follicular carcinomas (OC), 3 oncocyctic adenomas (OA) and 5 goiters (G).

For the immunohistochemical study, 4- μ m thick sections of buffered-formalin-fixed and paraffin-embedded tissues were used. The sections contained both tumoral and adjacent normal thyroid tissue.

The tissues were tested for nuclear expression of menin using an anti-menin antibody (Menin, A300-105A, Bethyl Laboratories, Inc., Montgomery, Texas, USA), at a dilution of 1:750 in an automatic system (Ventana) and a streptavidin-biotin/ peroxidase technique was used to visualise the labelling. All slides were stained with diaminobenzidine and then counterstained with haematoxylin. The nuclear semi-quantitative expression of menin was reviewed by the author and an experienced pathologist. If the expression in the tumour cells was equal to or higher than that observed in the normal thyroid tissue, it was considered “positive” expression, while if it was lower, it was considered “decreased” expression.

Table 1. Characteristics of the patients and the tumours with decreased expression of menin

Tumour	Age/Sex	Size (cm)	TNM stage	Mitosis
PDTC1	85/ F	NA	NA	4/10 HPF
PDTC2	56/ M	9.5	pT3N0M1	4/ 10 HPF
PDTC3	54/ M	7	pT3NxMx	1-2/ 10 HPF
PDTC4	78/ M	9	pT3N1bM0	4/ 10 HPF
OC1	42/ F	3.9	pT2N0Mx	0
OC2	74/ F	3.5	pT2N0Mx	0
PTC1	68/ F	0.8	pT1aNxM0	0
PTC2	26/ M	5	pT3N0Mx	0

*HPF- high power field; PDTC-poorly differentiated thyroid carcinoma; OC - oncocyctic follicular carcinoma; PTC-papillary thyroid carcinoma; NA - non available.

Statistical analysis

Data were analyzed in Microsoft Office Excel 2007®, and the results were expressed as median with range values.

RESULTS

The patients included in the study (21 males and 27 females) had a median age of 55 years (range 11-86).

The 5 patients with goiters had a median age of 35 years (range 23-53) and 60% were males. The expression of menin was positive and preserved in all 5 cases (Fig. 1.A). The 3 cases of oncocyctic adenomas belonged to 3 women with a median age of 54 years (range 50-60), had a median diameter of 3 cm (range 1.9-3.5), and all had a positive expression of menin. The 12 patients with papillary thyroid carcinoma had a median age of 40 years (range 18-68), and 33% of them were females (n=4). The thyroid tumours were classified as pT1 in 2 cases, pT2 in 6 cases, and pT3 in 4 cases. Median tumour diameter was 3 cm (range 0.8-5.8). Four PTC had lymph node involvement. The expression of menin was positive in all cases of PTC except for 2 (17%) where it was weakly expressed compared to the normal surrounding thyroid tissue (Figure 1.B). The two tumours, follicular variants of PTC, were pT1 and pT3, respectively. The characteristics of these tumours are showed in Table 1.

The 12 patients with poorly differentiated thyroid carcinoma had a median age of 63 years (range 43-85), and 58% were males. The tumours had a median diameter of 6 cm (range 3-9.5) and were pT2 in 1 case, pT3 in 9 cases, and pT4 in 2 cases. Three tumours had lymph node involvement and one developed in the mediastinum. From 12 PDTC, only 4 showed a decreased nuclear expression of menin, while the others presented a positive expression of menin. The characteristics of these 4 tumours with decreased expression of menin are presented in Table 1. All 4 tumours presented necrosis and convoluted and retracted nuclei. The mitosis varied from 1-2 to 8/ high power field (HPF). In all of them, the poorly differentiated contingent with decreased expression of menin was found on a background of other types of thyroid carcinomas, which all had a positive expression of menin, as follows: 1) conventional papillary thyroid carcinoma, pT4 (Fig. 2.A), 2) angioinvasive follicular carcinoma, pT3, 3) follicular variant of PTC, pT3 and 4) conventional variant of PTC, pT3 (Fig. 2.C). None of these tumours presented anaplastic contingent. One

Figure 1. Positive immunohistochemical expression of menin protein in the nuclei of different thyroid tumours (x10 magnification). A. Goiter (inlay 20x); B. Papillary thyroid carcinoma with oncocytic cells (inlay 40x); C. Medullary thyroid carcinoma (inlay 20x); D. Anaplastic thyroid carcinoma (inlay 40x).

case of poorly differentiated thyroid carcinoma, pT4, with no extrathyroid extension and no lymph node metastasis (Fig. 2.D) showed negative expression of menin. The tumour had a high index of mitosis (8/ 10 HPF), extensive necrosis, important anisokaryosis, and a 20% anaplastic carcinoma component due to which the tumour was considered pT4.

Of 5 patients with oncocytic follicular carcinomas, 3 minimally invasive and 2 widely invasive, 4 were females and had a median age of 65 years (range 39-74). The tumour size had a median of 4 cm (range 0.9-4.2), 3 tumours were pT2, and 2 were pT3. No lymph node involvement was noted. Three tumours showed a positive expression of menin and 2 cases a decreased expression of menin. These tumours had 3.9 and 3.5 cm respectively, both were pT2, the first one was minimally invasive and the second one widely

invasive. The tumours presented no necrosis or mitosis, and did not show extra-thyroidal extension (Fig. 2.B).

All the 6 cases of anaplastic thyroid carcinoma were positive for menin (Fig. 1.D). The tumours had a median diameter of 7 cm (range 1.5-10), and were classified as pT4, 3 of them with lymph node involvement. The patients had a median age of 68 years (range 58-86) and 67% were males.

In the 5 medullary thyroid carcinomas, the expression of menin was positive with no exception (Fig. 1.C). The tumours had a median diameter of 2 cm (range 1.1-2.2) and were pT1 in 2 cases, pT2 in one case, and pT3 in 2 cases. The patients had a median age of 62 years (range 11-66), 40% were males, and in 2 cases the medullary thyroid carcinoma was associated with multiple endocrine neoplasia type 2.

Globally, of 48 cases, 39 (81.25%) had a

Figure 2. Immunohistochemical expression of menin protein in the nuclei of different thyroid tumours. Pictures are taken with a 10x magnification, with different magnifications in the inlays. A. Follicular variant of papillary thyroid carcinoma with positive expression of menin (upper inlay 40x) and a poorly differentiated component with decreased expression of menin (lower inlay 20x); B. Oncocytic follicular carcinoma with decreased expression of menin (inlay 20x); C. Poorly differentiated thyroid carcinoma with decreased expression of menin (inlay 20x); D. Poorly differentiated carcinoma with absent expression of menin (inlay 20x).

positive expression of menin, while a decreased expression of menin was observed in 8 cases (16.6%). These tumours corresponded to 17% of the total number of papillary thyroid carcinomas, 33% of the total poorly differentiated thyroid carcinomas, and 40% of the total number of oncocytic follicular carcinomas.

DISCUSSION

The thyroid gland is not routinely involved in the Multiple Endocrine Neoplasia type 1. Nevertheless, data from literature are conflicting, since not all the studies collect information about the thyroid pathologies in MEN1 syndrome.

In the study of Marx *et al.*, they report a 12% incidence of thyroid disorders in a cohort of 130 patients

with MEN1 syndrome (9). From these patients, 8% had follicular adenomas and 5% PTC, with no detail over the PTC variant. In a recent study regarding the incidence of thyroid pathology in MEN1 patients *versus* non-MEN1 patients, the incidence of thyroid disorders did not differ between the two groups. MEN1 patients did not seem to have a higher incidence of thyroid tumours due to mutations in the MEN1 gene *versus* patients without MEN1 syndrome. They report that from the total cohort of 95 MEN1 patients, 45% presented a thyroid incidentaloma *versus* 51% in the non-MEN1 group. A histological subgroup analysis in 17 MEN1 patients revealed that the most frequent thyroid diseases were represented by follicular adenomas (n=4) and nodular dysplasia (n=5), followed by nodular hyperplasia (n=3), multinodular goiter (n=2) and one

case of multifocal microinvasive medullary thyroid carcinoma (MTC), microinvasive FTC and lymphocytic thyroiditis, respectively (10). In the same study, menin was tested for positive immunohistochemical staining in 5 thyroid tumors (hyperplastic nodule, microinvasive MTC, microinvasive FTC, multinodular goiter and follicular adenoma). Since the expression of menin was preserved in these thyroid tumors, the remaining copy of the MEN1 gene is capable of producing a normal menin protein.

Loss of heterozygosity (LOH) studies have identified several chromosomal sites harboring tumor suppressor genes involved in thyroid tumorigenesis. Since MEN1 syndrome does not usually involve thyroid, several reports have searched for a LOH in the second copy of the MEN1 gene which could lead to a total inactivation of the gene and a loss of normal expression of the oncosuppressive protein, menin. Nevertheless, none of the studies have identified such LOH at the MEN1 gene locus on chromosome 11 (10–12).

The study of Ward *et al.* regarding the LOH in sporadic thyroid tumors not related to MEN1 syndrome, found a LOH on chromosome 11q in 33% (3/9) of FTC and 6.25% (1/16) of PTC, a region including the MEN1 gene (13).

In 1999 Nord *et al.* analyzed 60 sporadic follicular thyroid tumors and identified that in 20% of the tumors (2/18 adenomas, 4/15 atypical adenomas, 1/6 Hurthle adenomas, 1/9 follicular carcinomas, 3/66 Hurthle carcinomas, 1/6 anaplastic carcinomas) there was a LOH in chromosome 11q13 where the MEN1 gene is located. The same authors analyzed the existence of mutations in MEN1 gene but genetic analysis did not show any mutations, only 2 polymorphisms which could not relate the development of the follicular neoplasms to the oncosuppressive properties of MEN1 gene (12). Therefore, they supposed that in this region of 11q13 could be another oncosuppressive protein, other than MEN1 gene responsible for thyroid tumorigenesis. Another study identified regions of loss on chromosome 11q in 17% (2/12) follicular adenomas and 10% (2/20) follicular carcinomas along with other chromosomal losses in the same tumors (14).

Our results show that, generally, menin expression is preserved in well differentiated thyroid carcinomas, but in poorly differentiated tumours the expression can be reduced and rarely absent.

The expression of menin was preserved in the majority of PTCs showing that in this type of well differentiated thyroid carcinoma, the MEN1 gene

does not seem to influence the tumorigenesis. Our observation is supported by cBioPortal database for Cancer Genomics which contains 123 cancer genomic studies and offers an insight in the oncogenes and their mutations (15). In this portal, the rate of MEN1 gene mutations in a subset of 511 papillary thyroid carcinomas is very low (0.2%, 1 case).

A high frequency of Ras point mutations was also reported in the follicular variant of papillary thyroid carcinomas (16,17). Another gene expression study demonstrated that cyclin D2 (CCND2), protein convertase 2 (PCSK2), and prostate differentiation factor (PLAB), allow an accurate molecular classification of thyroid carcinomas with follicular architecture, including FTC, follicular adenomas, and the follicular variant of PTC (18), which means that the oncogenic profile of these types of tumours could be similar. Of the 12 papillary thyroid carcinomas, only 2 presented a decreased expression of menin, the two cases corresponding to follicular variants of PTC. We suppose that our results could be explained by a possible link between the tumorigenesis of follicular tumours and menin inactivation, but further molecular studies are required to support this hypothesis.

Regarding follicular thyroid carcinomas, the molecular pathogenesis is still controversial. Oncogenic activation due to Ras point mutations is present both in follicular adenomas and carcinomas (17,19), including the oncocyctic follicular carcinoma (20,21). This oncogene is involved in the cellular signal transduction and mutations induce a continuous proliferation of cells, inevitably leading to cancer. In our study, menin expression was decreased in these type of carcinomas, suggesting that there could be a possible link between menin and the mitogen activated protein kinases (MAPK) pathway activated by the Ras mutations (20). The decreased expression of menin in the oncocyctic follicular carcinoma could also be explained by the observations of Kasaian *et al.* who reported somatic mutations with loss of function in the MEN1 gene in 4% of the tumours from a series of 72 oncocyctic follicular carcinoma (22). On the contrary, in a study of 27 oncocyctic follicular carcinomas, no somatic mutations were reported in the region of chromosome 11 where the MEN1 gene is located (20) thus leading to controversial results regarding the expression of menin and tumorigenesis in these types of thyroid tumours.

Poorly differentiated thyroid carcinomas are rare tumours in which the most prevalent genetic feature concerns Ras oncogene mutation (23). In our study, 33% (4/12) of the total number of poorly differentiated

thyroid carcinomas presented a decreased expression of menin, and one tumour did not express the protein at all. Kim *et al.* conducted a study on NIH3T3 fibroblast cell line modified to express the Ras oncogene (24). The research team transfected purified MEN1 plasmid DNA in stably transformed cells with oncogenic murine Ras, and evaluated the cell transformation. They observed that cultures containing the Ras oncogene contained abnormal cells, while the stable overexpression of menin suppressed the Ras-mediated phenotype (24). The study of Bertolino *et al.*, using MEN1 knockout mice, proved that the lack of menin expression is not compulsory for survival and cell proliferation, but it influences the differentiation of the cells and accelerates the senescence. This observation could indirectly explain the lack and/or decreased expression of menin in poorly differentiated thyroid carcinomas.

Interestingly, anaplastic thyroid carcinomas had a positive expression of the menin as well. Mutations of the p53 oncogene are commonly found in this type of tumour. The possible association of menin with tumour suppressive protein p53 was studied by Löffler *et al.* who reported that menin and p53 have additive effects and non-synergistic ones. They demonstrated that simultaneous heterozygous deletion of MEN1 gene in animals with either heterozygous or homozygous deletion of Trp53 gene did not result in formation of tumours at any new sites. In addition, mice retained both of the tissue specific patterns of tumorigenesis associated with each of these tumour suppressor proteins (25). In our study, the expression of menin was preserved in the anaplastic thyroid carcinomas, which proves the fact that the increased aggressiveness of these tumours is not related to the expression of menin but to other molecular mechanisms.

Since menin is known to be localized in the nucleus of non-dividing cells, and that in dividing cells it is shuttled in the cytoplasm (26,27), we could suppose that the lack/decreased nuclear menin expression in poorly differentiated thyroid carcinomas could be caused by the increased index of mitosis seen in these tumours. On the contrary, in anaplastic thyroid carcinomas which have also higher mitotic index, the nuclear expression of menin is preserved. However, we cannot exclude the possibility that molecular pathways in this type of thyroid carcinomas could result in changes of the normal behaviour of menin.

Nevertheless, we cannot exclude the fact that the negative expression of menin could also be related to somatic mutations in the MEN1 gene, leading to an inactive truncated protein, no longer recognized by the

epitope of the antibody used for immunohistochemistry. To date, 1336 MEN1 sequence gene abnormalities have been reported (1133 germline and 203 somatic mutations) (28–30). This inactivation is caused by small mutations in the reading frame of the gene, involving one or several bases or it can be a de novo germline mutation.

Medullary thyroid carcinomas occur in both sporadic and hereditary forms as part of multiple endocrine neoplasia type 2. Germline or somatic mutations in the RET oncogene localized on chromosome 10q11-2 are found in almost all hereditary cases (31). We noted a positive expression of menin in all our tumour samples, showing that in these cases, the MEN1 gene does not influence the development of MTC.

In conclusion, the results of our study show that menin expression is generally preserved in different types of thyroid carcinomas, with a decreased expression in follicular carcinomas, poorly differentiated thyroid carcinomas and follicular variant of papillary thyroid carcinoma. Further molecular studies are required to better understand the impact of menin protein in thyroid tumorigenesis.

Conflict of interest

The authors declare no conflict of interest.

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