PhD thesis
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THE ROLE OF MENIN IN THE THYROID CARCINOMA

Abstract

Thyroid carcinomas are the most frequent endocrine neoplasms in humans, with an incidence of ~ 1.5%. The incidence has increased in the last decades, especially after the 1986 Chernobyl nuclear power plant disaster and also due to the increased use of neck ultrasound and advanced imaging techniques which allow the incidental diagnosis of thyroid pathologies. Despite their increased incidence, thyroid cancers are slow growing tumours with a loco-regional extension and usually of a good prognosis. Multiple Endocrine Neoplasia type 1 (MEN1) is a hereditary syndrome with an autosomal dominant transmission characterized by the development of hyperplasia and tumors in certain endocrine glands. The diagnosis is made in the presence of an association of at least 2 endocrine tumors. The gene involved in the development of MEN1 syndrome is MEN1 and encodes an oncosuppressive protein, *menin*. Data from the literature regarding the involvement of thyroid pathologies in MEN1 syndrome are controversial. The studies indicate that thyroid pathologies are present from 2.6% up to 25% in the MEN1 syndrome. The variation of the percentage is due to the lack of systematical collection of data regarding thyroid pathology in this syndrome.

The aims of the present thesis are to evaluate if the selective inactivation of MEN1 gene could induce a more aggressive thyroid tumorigenesis in murine models and to evaluate if the expression of the protein is altered in different types of human thyroid carcinoma.

The general part of the PhD thesis presents the actual knowledge of thyroid cancer incidence, with an insight in the oncogenes involved in tumorgenesis along with the role of *menin* in this pathology. In addition, this part presents the most important murine models prone to develop thyroid carcinomas with a description of the induced thyroid lesions. The second part of the thesis presents the personal contribution to this subject and consists in two studies.

The hypothesis of the first study emerged from the observations that knock-out mice, with complete inactivation of MEN1 gene in all the tissues, develop at advanced ages, different thyroid lesions, some corresponding to thyroid cancer. The objective of the first study was to evaluate if the selective inactivation of *menin* in the murine thyroid glands expressing *RET/PTC3* or *E7* oncogenes, might induce a more rapid development of thyroid hyperplasia and/or tumors along with an increased index of proliferation.

We analyzed the thyroid glands of 77 mice aged 4–18 months. The mice were divided in 9 groups: wild type mice (without the expression of any oncogenes and without inactivation of *menin*), mice with selective homo- or heterozygous inactivation of *menin* in the thyroid gland and no expression of other oncogenes, mice that express the RET/PTC3 oncogene without inactivation of *menin*, mice that express the RET/PTC3 oncogene with selective homo- or heterozygous inactivation of *menin* in the thyroid gland, mice that express the E7 oncogene without inactivation of *menin* and mice that express the E7 oncogene with selective homo- or heterozygous inactivation of *menin* in the thyroid gland. In total, 52 mice presented selective inactivation of *menin* in the thyroid gland (16 in the group of mice expressing the RET/PTC3 oncogene, 19 in the group of mice expressing the E7 oncogene and 17 from the group of mice not expressing any oncogenes). We analyzed general aspects regarding the weight of the mice and the thyroid gland, the surface of the thyroid gland, Ki67 proliferation index in the thyroid gland, cellular density and histopathological changes in the thyroid gland.

As compared to the wild type mice, those with selective inactivation of *menin* presented an increased Ki67 proliferation index and cellular density, without histopathological changes in the thyroid gland or tumour development. Mice expressing the

E7 oncogene had the largest thyroid glands with a pattern of diffuse hyperplasia but no tumour development and no changes in the cellular density. Mice expressing the *RET/PTC3* oncogene presented larger thyroid glands compared to the wild type mice but smaller compared to *E7* mice. The Ki67 index of proliferation and the cellular density were the largest from all the groups but did not vary according to the status of *menin* inactivation. The lesions in the *RET/PTC3* group were the most heterogeneous and varied from "proliferative papillary cystic changes" in 60% of cases, "cribriform" pattern in 16% of cases, to "solid" pattern in 8% of cases and a combination of these patterns in the rest of the thyroid glands without a particularity of distribution of the lesions according to the *menin* status. We observed that in both groups expressing the RET/PTC3 and E7 oncogenes with selective homozygous inactivation of *menin*, the thyroid surface was more reduced as compared to the mice expressing the oncogenes but without inactivation of *menin.*

Globally, our study showed that the selective inactivation of *menin* in the thyroid glands of mice not expressing other oncogenes does not induce thyroid tumorigenesis, but it influences the thyroid cellular proliferation and density, with an increase in the Ki67 index of proliferation. In the case of mice expressing both oncogenes, the selective inactivation of *menin* determined a reduced thyroid surface but did not influence the Ki67 index of proliferation or the cellular density. In addition, in these groups we did not notice any thyroid tumour formation but the histological changes induced by the oncogenes have an uncertain degree of malignancy. These lesions could mimic the poorly differentiated thyroid carcinoma or the diffuse sclerosing variant of papillary thyroid carcinoma.

The hypothesis of the second study was that in poorly differentiated and anaplastic thyroid carcinomas, the nuclear expression of *menin* could be altered. The objective of this study was to analyse the immunohistochemical expression of *menin* in different types of thyroid carcinomas not related to MEN1 syndrome. For this study we evaluated 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 oncocytic follicular carcinomas (OC), 3 oncocytic adenomas (OA) and 5 goiters (G)) for nuclear expression of *menin* using an anti-menin antibody. The expression was considered positive (similar to that of the normal thyroid tissue), negative or decreased as compared to the normal thyroid tissue. The expression of *menin* was positive, identical to the 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. Our results show that the expression of *menin* is generally preserved in human thyroid carcinomas, but it can be decreased or absent in the follicular carcinomas, follicular variant of PTC and PDTC. Another observation is that the expression of *menin* does not correlate with the degree of aggressiveness of the human thyroid tumours since its expression was positive in anaplastic and medullary thyroid carcinomas.

The originality of this thesis consists in the presentation of the morphological and histological aspects induced by the selective inactivation of *menin* in the thyroid gland of transgenic mice, with the observation that up to the present moment there are no similar studies, with the exception of those presenting the effects of inactivation of *menin* in knock-out mice for MEN1 gene. In addition, the present thesis evaluates the expression of *menin* in a significant cohort of different human thyroid carcinomas. The role of *menin* as an oncoprotein involved in the thyroid tumorigenesis still remains the subject of research, especially molecular studies, in order to further evaluate its role in the induction of thyroid carcinoma.

Keywords: thyroid carcinoma, menin, RET/PTC3 oncogene, E7 oncogene