The natural history of thyroid autonomy and hot nodules

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Évolution naturelle des nodules chauds et de l’autonomie thyroïdienne

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Ann. Endocrinol., 2003 ; 64, 1: 17-22

L’adénome thyroïdien autonome est une tumeur monoclonale bénigne dont la croissance et la fonction ont échappé au contrôle hypophysaire ; la sécrétion d’hormones thyroïdiennes n’est dès lors plus contrôlée. La survenue d’un adénome autonome est le plus souvent attribuable à une mutation activatrice du récepteur de la TSH. À la scintigraphie, ces nodules apparaissent comme chauds ou hypercaptants car le nodule thyroïdien concentre de manière préférentielle ou exclusive le radioiode ou le 99mTc pertechnate, le reste du parenchyme étant partiellement ou complètement éteint. Le mode évolutif est probablement le suivant : d’abord présence d’un petit adénome autonome initialement isocaptant ou légèrement hypercaptant par rapport au tissu sain. On parle alors d’adénome prétoxique. Ensuite, avec l’augmentation de la taille de l’adénome, augmentation progressive de la synthèse d’hormones thyroïdiennes avec diminution progressive du taux de TSH et extinction concomitante du parenchyme sain. Le risque d’évolution d’un adénome prétoxique vers un adénome toxique est de l’ordre de 4 % par an et va dépendre de l’âge du patient, de la taille de l’adénome et de l’apport iodé. Il n’y a pas de relation claire entre le phénotype de l’adénome et les différentes mutations du récepteur TSH.

Mots-clés : Hyperthyroïdie, nodule chaud, autonomie thyroïdienne, récepteur TSH.

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Solitary hyperfunctioning thyroid adenomas are benign monoclonal tumors characterized by their capacity to grow and produce thyroxine (T4) and triiodothyronine (T3) autonomously, i.e. in the absence of thyrotropin (TSH) [6, 24, 25]. Thyroid nodules are classified according to their ability to take up the isotope compared to that of the extranodular tissue. A cold nodule (hypofunctional) has reduced tracer uptake, a warm nodule (isofunctional) has tracer uptake roughly equivalent to the nonnodular tissue and a hot nodule (hyperfunctional) has increased tracer uptake. Approximately 85% of all nodules are cold, 10% are warm and 5% are hot [21]. Beside this isotopic classification, a hot nodule is called “toxic” when there is evidence of biological or clinical hyperthyroidism. On radioisotope scanning they generally appear as hot nodules because they concentrate radioiodide or 99mTc pertechnate, whereas the normal surrounding and contralateral tissue concentrate little isotopes because it is progressively deprived of its normal tonic TSH stimulation. A toxic adenoma probably evolves gradually from a small autonomously hyperfunctioning adenoma that initially is only slightly more active than the extranodular tissue. This has been referred to as a “warm” nodule or a “compensated” adenoma. The diagnostic criterion for this designation is the persistence of detectable serum TSH maintaining some radioiodine uptake by the extranodular tissue. This “compensated” adenoma persists as long as the autonomous hormone output is not sufficient to suppress thyrotropin, i.e. to cause hyperthyroidism. The rate of development of thyrotoxicosis in patients with hyperfunctioning adenomas who are euthyroid initially is about 4% per year and depends on the size of the adenoma, iodine intake and age of the patient. No clear relationship can be established between the nature of the TSH receptor mutations and the phenotype of the tumor.

Key words: Hyperthyroidism, hot nodule, thyroid autonomy, TSH receptor.
utes little to overall thyroid secretion. As the adenoma grows, its contribution to thyroid secretion increases and TSH secretion therefore decreases. With further growth, thyroid secretion becomes supranormal, and TSH secretion becomes unequivocally low, so that the extranodular tissue takes up little if any radionuclide, and the patient develops overt thyrotoxicosis [12].

In a patient with a thyroid nodule, the finding of a low serum TSH concentration indicates the presence of a hyperfunctioning adenoma. In some cases, a hyperfunctioning adenoma of the thyroid that does not produce hyperthyroidism may be recognized. This has been referred to as a “warm” nodule or a “compensated” adenoma. The diagnostic criterion for this designation is the persistence of detectable serum TSH maintaining some radioiodine uptake by the extranodular tissue. This results in a more uniform radioiodine scan, with an uptake slightly higher or equal in the nodule than in the normal surrounding tissue. This “compensated” adenoma per-
sists as long as the autonomous hormone output is not sufficient to suppress thyrotropin, i.e. to cause hyperthyroidism. It is likely that a warm nodule precedes the appearance of a toxic adenoma. There are however few reports of prolonged observations of patients with a warm nodule confirming the progression to true toxic adenoma. The autonomous function can be demonstrated by administration of suppressive doses of T3 which does not affect the function of the nodule (persistence of radionuclide uptake) but decreases uptake by extranodular tissue. Conversely TSH administration increases or restores radionuclide uptake in the quiescent tissue [13].

**EPIDEMIOLOGY**

The prevalence of solitary toxic adenoma as a cause of thyrotoxicosis varies throughout the world. In the United States these adenomas account for only 2% of cases of thyrotoxicosis, whereas the prevalence is higher both in South America and Europe; in some areas of Switzerland and Germany they account for more than 30% of cases of hyperthyroidism [1, 12, 17, 20]. A prospective survey in six different European countries suggest that the frequency of autonomous adenomas in a population is inversely related to iodine intake; the prevalence was more than three times higher in areas in which iodine was relatively low, as compared with areas in which iodine uptake was higher [20]. In a retrospective study in Sicily, a higher percentage of autonomous thyroid nodules was also reported in iodine deficient areas [2]. The key role of iodine supply in the pathogenesis of autonomous thyroid nodules is also suggested by the fact that in Switzerland they became less common after iodine intake increased [1]. Autonomous adenaoma can occur at any age but is generally diagnosed between 30 and 60 years of age. Like most thyroid diseases, autonomous nodules are more common in women, with a female: male ratio ranging from 6:1 to 15:1 [2, 12, 13].

**PATHOGENESIS OF AUTONOMOUS ADENOMAS**

Hyperfunctioning adenomas, including those that cause thyrotoxicosis, are clonal lesions of thyroid follicular cells. Their metabolic characteristic are homogenous [24]. The monoclonality of toxic adenomas is demonstrated by different methods [14, 16]. The same mutation is found in all its cells; many have somatic activating mutations of TSH-R some have mutations of the α subunit of the guanine nucleotide-binding (G) protein (Gsα). Monoclonality implies that the adenoma derives from the successful proliferation of one initial cell in which the causal mutation has occurred. Whether other additional genetic or epigenetic events contribute to the later development of the lesion remains an open question. This presumed derivation from a single cell allows estimation of the minimal number of divisions necessary to achieve a clinically apparent adenoma. Ten divisions without cell loss multiplies cell number by $2^{10}$ i.e. about 1000. Assuming a mean cellular volume of 1 picoliter, a lesion of 1 ml or 1 g would require a minimum of 30 divisions. The shortening of telomeres in the chromosomes of adenomas is compatible with this estimate [7]. Considering that a normal thyroid cell in an adult divides about 5 to 7 times during adulthood, this represents a striking increase in the apparent in vivo lifespan of thyrocytes. At the time of surgery, the Ki67 index in toxic adenomas (a histochemical marker of cells engaged in the mitotic cycle) is increased by a factor of 4, as compared with normal thyroid tissue, which, in the absence of cell loss, would correspond to a doubling time of about 2 years [8]. This labelling is heterogeneous, with a greater intensity at the periphery of the tumours, and varies greatly from one adenoma to another. There is therefore a profound discrepancy between the rate of cell division necessary to generate a visible tumour (30 divisions over a few years) and the rate of cell division observed at the time of surgery (maximum one division every two years [8]). The conclusion is that by the time the adenoma becomes clinically detectable and surgical excision is deemed appropriate, the growth rate is slow [2, 12, 13], a conclusion confirmed by the lack of change in those patients who are followed without treatment. The adenoma cells are therefore at the end of their proliferation process [13]. This stationary state may theoretically result from an equilibrium between cell division and apoptosis but in one study, no apoptosis was observed in the adenoma as studied by the TUNEL method [8]. The data therefore show that the proliferation characteristics of autonomous adenomas and perhaps other biochemical aspects, are not constant throughout the life of the lesion.

The prevalence of TSH-R mutations in toxic adenomas has varied considerably in different studies. How lower iodine intake may contribute to mutations in the TSH-R is not known. In iodine deficient areas, thyroid glands are exposed to a mild chronic stimulation by TSH. This stimulation might create a mutagenic environment caused by TSH dependent generation of H$_2$O$_2$, generation and secondary production of free radicals [3, 9]. TSH, by increasing the cell replication rate, will fixate more mutations before repair and therefore increase the frequency of established mutations. It is also possible that the degree of iodine deficiency may modulate further evolution of the mutated cell and the emergence of a phenotype [5].

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CLINICAL EVOLUTION

The evolution of a hyperfunctioning adenoma to the stage of a toxic adenoma is gradual, and is not inevitable. Thyrotoxicosis is rare in patients with adenoma less than 2.5 cm in diameter or less than 5 ml in volume; in contrast, about 80% of patients with adenomas more than 5 cm in diameter have thyrotoxicosis [12]. Among all patients with hyperfunctioning adenomas, more than 75% are euthyroid when their adenoma is first detected [12], [22]. Most of these nodules do not substantially change in size with time. In a study in the United States in which 287 patients were followed for 1 to 15 years [22], the size of the adenoma increased by 1 cm or more in only 9%, there was no change in 86%, and the adenoma decreased in size in the remainder. Any decrease in size is probably due to hemorrhagic infarction.

The rate of development of thyrotoxicosis in patients with hyperfunctioning adenomas who are euthyroid initially is about 4% per year [12, 22, 23]. The risk of thyrotoxicosis is higher in older patients, those in whom the adenoma is 3 cm or more in diameter at the time of the diagnosis, and those living in areas of low iodine intake [2]. In a patient with a hyperfunctioning adenoma, iodine supplementation may precipitate thyrotoxicosis. In a study of Ermans et al, four euthyroid subjects with a hyperfunctioning adenoma became thyrotoxic when they were given 0.5 mg iodine per day [11]; in another study of similar patients, administration of 0.1 or 0.2 mg iodine resulted in substantial increases in serum T4 concentrations [18].

MECHANISM OF THE HYPERTHYROIDISM

The amount of thyroid hormone secretion by autonomous adenomas can be evaluated from the product of the following formula: (number of cells) x (activity of individual cells) x (iodine supply). The first variable can be estimated by the volume of the tumor, taking into account that cell number per unit volume may vary by a factor of 2. Iodine supply can be estimated at equilibrium by the daily urinary excretion. The activity per cell can only be estimated from the ratio (thyroid hormone secretion) / (volume x iodine supply). The relationship of the incidence of hyperthyroidism to the volume of the nodule and the induction of thyrotoxicosis by iodine administration (vide supra) support this reasoning. Hyperthyroidism can only occur for a volume of at least 5 ml i.e. one half the normal thyroid volume. It can therefore be estimated that the activity of the cells cannot be increased by more than by a factor of 2 [9]. Whether activity per cell is constant from one nodule to another remains an unanswered question. If it was, it would mean that, for a given iodide supply, hyperthyroidism would systematically appear when the nodule reaches a given size. A good correlation is reported in the literature between the size of the nodule and the risk of thyrotoxicosis in a given area, however some patients are thyrotoxic for a nodule of less than 2.5 cm in diameter whereas some are not for a diameter above 5 cm. This heterogeneity may reflect the heterogeneity of cellular density from one nodule to another due to various decreases in colloid space or the difficulty of estimating the actual weight of the active nodule particularly in the presence of partial necrosis. As daily urinary excretion was not measured, it may also reflect different iodide supply. However, when it was clear that activating mutations of the TSH-R was a major cause of toxic adenomas, it was tempting to speculate that the strength of the activation of adenylate cyclase that varies from one mutation to another may lead to different activity per cell but also different iodide uptake and rate of growth and therefore different phenotypes.

GENOTYPE-PHENOTYPE RELATIONSHIPS

The functional studies of the various TSH-R mutants were performed in transfected COS cells, a system in which very high concentrations of receptors are achieved. While this allows unmasking functional characteristics of the mutants within a few days, it poses the question of the clinical relevance of the observations made in these ex-vivo experiments. One potentially interesting point relates to the capacity of only a few of the mutants to activate constitutively both the cAMP and the inositolophosphate-diacylglycerol- Ca\(^{2+}\) (IP-DAG-Ca\(^{2+}\)) regulatory cascades. In the human thyroid gland, cAMP activates iodide trapping and T4 and T3 secretion, while the Ca\(^{2+}\) regulatory pathway is implicated in the control of H2O2 generation and iodide oxidation and organization (T4 and T3 synthesis) [4]. Accordingly, adenomas with TSH-R mutants that activate only the cAMP cascade would be expected to synthesise less T4 and T3 than adenomas with mutant TSH receptors that activated both pathways. However, differences between the effects of the mutants in COS cells and thyrocytes in vivo may render these kind of correlation a difficult exercise.

Besides different activity per cell, we can also imagine that different genotypes may lead to different growth rates. However this parameter is difficult to analyze as, when diagnosed, the adenoma is at the end of its proliferation process and the time interval between the primary mutation and the appearance of a symptomatic adenoma is not known. Because of the rarity of individual mutations and the limited size of se-
ries investigated, no systematic study has yet been performed to establish a correlation between the nature of the mutations and the phenotype of the tumors. However no obvious differences were found among the clinical records of cases harboring different mutations, particularly between those harboring mutation activating both regulatory cascades and those with only stimulation of cAMP accumulation. It is therefore likely that parameters other than the nature of the mutations may govern the course of the disease. The fact that in hereditary toxic thyroid hyperplasia (HTTH) the age of onset may vary from infancy to adulthood even among the members of the same family harboring the same mutation suggest the influence of other parameters [10]. These differences in the expressivity of mutations may reflect differences in the genetic background of the affected individuals as well as differences in environmental factors like the amount of iodine or goitrogens in the diet. However, comparing the spectrum of somatic mutations found in toxic adenomas with germline mutations found in HTTH leads to the observation that they only partially overlap. The familial cases harboring a mutation in common with toxic adenomas have a more severe phenotype with an earlier hyperthyroidism onset than the others. A likely explanation is that this reflects a selection bias. Starting as a single cell, to produce a sizable tumor, the adenoma type mutation must be more aggressive to be selected by the clinical screening. In comparison, mutations causing a milder stimulation are expected to cause hyperthyroidism only if expressed in all thyrocytes during many years as in most familial cases.

There is therefore no obvious relationship between the activating mutation of the receptor and the phenotype. This may be due to several reasons:

1) The difficulty in establishing a pure phenotype. The phenotype is generally defined by the severity of hyperthyroidism and the size of the adenoma. Various iodide intake may modify the severity of hyperthyroidism. A supplementation of only 100 to 200 μg (rougly the difference in iodine intake between the countries with a moderate shortage of iodine and the others) is sufficient to cause hyperthyroidism in euthyroid patients with autonomous nodule [18] As daily urinary excretion of iodine is generally not measured, different iodine intake may complicate the estimation of the intrinsic severity of hyperthyroidism. The size of adenoma may be in some circumstances a poor estimation of the total volume of active cells. It is possible that a better estimation of the activity per cell will improve the correlation with the nature of the causal mutation in the TSH-R. It is also possible that other parameters generally not investigated like the rate of growth correlates better with the causal mutation.

2) The possible irrelevance of comparing functional characteristics of mutants in COS cells with the activity of the adenoma bearing the mutant receptors in vivo. Intracellular feedback mechanisms may play a role in vivo and modulate the severity of the phenotype. For instance a secondary increase in cAMP degrading activity was described in thyroid cells from autonomous adenoma [19].

3) The possibility that chronic stimulation of the cAMP pathway in thyrocytes by the mutated receptor can lead to secondary unknown mutational or epigenetic events responsible for different phenotypes in patients harboring the same mutation. This is illustrated by the heterogeneity of clinical presentation in family of patients affected by HTTH. Secondary events may also be responsible for later nodular transformation of thyroid in patients with germline mutation [15].

4) The lack of data concerning the TSH-R number per cell in adenoma in vivo.

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