Follicular thyroid carcinoma

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Follicular thyroid carcinoma is being diagnosed less and less frequently despite the increasing incidence of well-differentiated thyroid carcinomas everywhere. This review will discuss the reasons underlying such an observation focusing on the evolution of the morphological and immunohistochemical diagnostic criteria of follicular thyroid tumors. It will address the differential diagnosis between follicular carcinoma and three tumor types—follicular adenoma, follicular variant of papillary carcinoma and poorly differentiated carcinoma—as well as the problems raised by the newly described categories of follicular tumors: follicular tumor of uncertain malignant potential, well-differentiated tumor of uncertain malignant potential and well-differentiated carcinoma, not otherwise specified. Finally, the prognostic and therapeutic significance of some promising molecular biomarkers will be discussed within the frame of the aforementioned histopathological classification.

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In the respective chapter of the *WHO Classification of Tumours of Endocrine Organs*, follicular thyroid carcinoma (FTC) is defined as ‘A malignant epithelial tumor showing follicular cell differentiation and lacking the diagnostic nuclear features of papillary thyroid carcinoma’ (PTC).¹ This definition is almost identical to the definition advanced by Rosai *et al*² in the AFIP fascicle on *Tumors of the Thyroid Gland*: ‘Follicular carcinoma is a malignant epithelial tumor showing evidence of follicular differentiation and not belonging to any of the other distinctive types of thyroid malignancy.’

Besides the point on follicular cell differentiation that does not usually raise any major difficulty, the two crucial issues of both definitions reside in the need to demonstrate malignancy and to exclude the presence of nuclear features typical of PTC.^{1–3}

In this context, malignancy is equivalent to invasiveness (capsular and/or vascular penetration), which may be difficult to disclose with certainty.^{1–3} The situation is even worse regarding the tendency

to overclassify the nuclear features of many follicular growth-patterned lesions as suggestive or indicative of those of the PTC nuclei; this trend is leading to an overdiagnosis of the follicular variant of PTC (FVPTC) and to an underdiagnosis of FTC.^{1,4}

The utilization of more strict criteria for recognizing capsular and vascular invasion, and the aforementioned trend toward overdiagnosing FVPTC has led, together with the increasing number of PTC diagnoses (namely small or very small lesions), to a progressive reduction in the percentage of FTCs among well-differentiated thyroid carcinomas.^{1–4} For instance, the percentage of FTCs among well-differentiated carcinomas decreased from 28.1% in the period 1983–1992 to 6.5% in the period 1993–2007 in a very large series from Greece.⁵ In Japan, Kakudo *et al*⁶ recently reported that FTCs represented 9.7% among well-differentiated and moderately differentiated carcinomas. In a multi-institutional study, we are performing in Porto, FTCs composed of ‘normal’ follicular cells or oncocytic cells represent ~5% of clinically evident well-differentiated carcinomas (Magalhães *et al*, unpublished results). At present, in most US institutions, the frequency of FTC is even lower than that reported in the aforementioned series.

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Regardless of the differences from series to series, it is generally acknowledged that *bona fide* FTC is nowadays a relatively rare thyroid tumor in contrast to its high frequency in the series of the first half of last century.^{1–4}

The prognosis of FTC depends on several factors: age of the patients, size and staging of the tumors, completeness of surgery and responsiveness to radioactive iodine.^{1–3,7} It also depends on the degree of invasiveness of tumors: minimally invasive carcinomas carry a much better prognosis than do widely invasive carcinomas.^{1–3,7} The prognostic importance of angioinvasion has been recently acknowledged.^{1,7,8} The prognostic meaning of tissue and cell differentiation remains controversial, although it is generally accepted that FTCs composed of oncocytic cells and FTCs with a solid, trabecular or insular growth pattern respond less well to radioactive iodine and may carry a worse outcome.^{1–3,7,9,10} The coexistence of less well-differentiated areas in some FTCs raises the problem of the differential diagnosis between FTC and poorly differentiated thyroid carcinoma (PDTC).^{1,7,11}

The above-mentioned diagnostic and prognostic issues will be addressed in this review using the chapter on follicular carcinoma of the WHO book,¹ not only as a frame but also as a starting point for consolidating the classic criteria and discussing some recently advanced proposals. The molecular features will be integrated in the respective clinico-pathological sections.

Macroscopy and invasiveness

Most FTCs are encapsulated solid tumors with a gray tan to brown color at the cut surface (Figure 1a). Minimally invasive FTCs are macroscopically indistinguishable from FA, except for capsular characteristics; the capsule tends to be thicker and more irregular in FTC than in FA.^{1–3,7} This is such an important feature that provided one is not dealing with a widely invasive FTC, the hypothesis of FTC should be ruled out if the tumor has no capsule or just a thin one. Widely invasive FTCs may occur as partially encapsulated tumors with extensive penetration of the capsule or as multinodular, bulking tumors without a capsule, occasionally showing permeation of the thyroid veins (Figure 1b).^{1–3,7}

FTCs usually occur as single tumors. Multifocality and recurrence in the residual parenchyma after partial thyroidectomy are uncommon. The aneuploid pattern and the various molecular alterations detected in FA and FTC^{12–15} support the assumption that the development of such tumors is the end product of multiple oncogenic steps, thus justifying their usual appearance as single (unicentric) neoplasms.¹⁵ At variance with FTCs, PTCs occur frequently as multicentric neoplasms, which may be clonally independent from each other;¹⁵ taking this together with the fact that almost every PTC is

Figure 1 Macroscopic appearance of the two main types of follicular thyroid carcinoma. (a) Minimally invasive follicular thyroid carcinoma. (b) Widely invasive follicular thyroid carcinoma.

diploid or quasi-diploid and display microsatellite stability, it is tempting to advance that, in contrast to FTC, PTC tumorigenesis reflects the end product of a few oncogenic steps.^{15–17}

The classification of FTCs in two categories—minimally and widely invasive—has always created a sense of a ‘pathological’ gap: what about tumors that are ‘simply’ invasive? This gap has been filled with the creation of a third group of FTCs, the angioinvasive carcinomas.^{1,7} FTC is at present classified as ‘minimally invasive without angioinvasion,’ ‘minimally invasive with angioinvasion’ and ‘widely invasive.’ Most authors agree that for practical (prognostic and therapeutic) purposes, it is crucial to separate minimally invasive, not angioinvasive, FTCs which carry an excellent prognosis from angioinvasive FTCs regardless of whether it falls in the category of widely invasive FTCs.^{1–3,7,18–20}

We believe that it is not worthwhile trying to count the number of vessels invaded by neoplastic cells in an attempt to establish a threshold separating two groups of prognostically different angioinvasive FTCs. Nikiforova *et al*²¹ have shown an association between the *PAX8-PPAR γ* rearrangement and very angioinvasive FTC ('overtly' invasive FTC), and a similar association was found by our group in FVPTC.²² These findings reinforce the molecular similitude of FA/FTC and FVPTC (see below) and support the putative pathogenic importance of the *PAX8-PPAR γ* rearrangement in different types of follicular-patterned tumors, but there is not (yet?) enough evidence to use the detection of the *PAX8-PPAR γ* rearrangement for prognostic purposes. This is because first, *PAX8-PPAR γ* rearrangements are relatively frequent in follicular adenomas,²³ and second, it is not known whether its presence carries prognostic information independently from vascular invasion.

In daily practice, we always try to detect at least one unequivocal focus of vascular invasion and, whenever this purpose is achieved, a diagnosis of angioinvasive FTC is made.²⁴ Incidentally, whenever one identifies a focus of vascular invasion, several others are usually detected in the same section or in deeper sections.

The morphological criteria for identifying, for sure, signs of vascular invasion have been thoroughly described by LiVolsi,³ Rosai *et al*² and Rosai,⁷ as well as in the WHO book.¹ Although we agree with Mete and Asa⁸ about the ideal criteria for diagnosing vascular invasion (tumor cells invading a vessel wall associated with the thrombus adherent to intravascular tumor), we diagnose vascular invasion if we detect well-preserved neoplastic issue within a vein (Figure 2). Whenever facing the dilemma 'vascular invasion or not,' we do not rely on any immunostaining of endothelial cells; we search the additional sections of the capsule and stick to the following rule: If we do not detect any unequivocal signs of venous permeation, we diagnose the tumor as nonangionvasive (see below the point on 'follicular tumors of uncertain malignant potential').

Histology and differential diagnosis

Histologically, FTCs display variable morphology ranging from small/medium-sized follicles containing colloid to trabecular or solid growth pattern (Figure 2). The latter tends to be more frequent in widely invasive FTCs, but they can also occur in minimally invasive FTCs. It is exceedingly rare for a FTC to be composed of macrofollicles; in case one faces a capsular and/or vascular invasive follicular tumor composed of large follicles, with or without colloid, it is mandatory to look for the nuclear characteristics of neoplastic cells because the most likely diagnosis will be a (macro) FVPTC.

The cytological aspects can also vary a lot from case to case, and even within each case, without any clear-cut distinction between minimally and widely invasive FTCs. The same holds true regarding FA and adenomatous nodules of multinodular goiters which may present, either throughout the nodule or in some foci marked as cyto-architectural atypias. In other words, neither architectural nor cytological atypia are reliable criteria of malignancy.^{1-3,7,24} This and the need to identify unequivocal signs of invasion to make a diagnosis of FTC, renders fine-needle aspiration cytology and frozen sections useless or almost useless in these settings.^{1-3,7}

Differential Diagnosis between FA/Adenomatous Nodule and Minimally Invasive FTC

Despite the huge amount of information on proliferation, apoptosis, cell-cycle differentiation and stromal markers obtained at the immunohistochemical level,^{1,15,25-31} the differential diagnosis between FA/adenomatous nodule and minimally invasive FTC still depends on the identification of histological signs of invasiveness.^{1-3,7,24} The search for such signs should be made in numerous H&E-stained sections involving the capsule of the tumor.^{1-3,7,24} In our hands, as stressed above, immunostaining of capsular vessels does not provide useful diagnostic data. There is also no immunohistochemical or molecular feature that may be used as a reliable marker of invasion.^{1,24,28}

The most prominent molecular features of FTC are the prominence of aneuploidy and the high prevalence of *RAS* mutations and of *PAX8-PPAR γ* rearrangements, the latter as a result of a 2;3 translocation.^{12-15,32-34}

All the aforementioned molecular features (such as aneuploidy, *RAS* mutations, *PAX8-PPAR γ* rearrangements, etc.) are also frequently observed in FA,^{12-15,22,23,32-35} and are therefore almost useless for diagnostic purposes. In addition, the molecular approaches for diagnosing malignancy in follicular tumors using high-throughput technologies (DNA microarrays, massive sequencing, etc.) did not provide diagnostically sound results.³⁶⁻³⁸ The interesting preoperative diagnostic findings reported by Cerutti *et al*³⁹ have not been confirmed in other series, and the same holds true for other studies.³⁸

Taking into consideration the recently reported complex landscapes of somatic rearrangements in human breast cancer genomes⁴⁰ and the more than

Figure 2 Histological appearance of a minimally invasive, angioinvasive, follicular thyroid carcinoma. (a) Microfollicular/solid pattern of growth of the neoplasia surrounded by a thick capsule (HE, $\times 4$), (b) displaying, focally, capsular invasion (HE, $\times 4$) and (c and d) vascular invasion (HE, $\times 100$).

20 000 or 30 000 somatic mutations detected in individual human cancers,^{41,42} it may be anticipated that rather than searching for mutations by massive sequencing, it seems more sensible to progress in the diagnosis and prognosis of thyroid tumors by an integrated histological and molecular approach.²⁸ The morphological frame is crucial for the topographical characterization of any tumor; this holds true particularly for follicular tumors of the thyroid as they epitomize the most important cancer properties: invasiveness in general and vascular invasiveness in particular. Besides 'topography,' the search for serum biomarkers may prove diagnostically

useful in this setting. Despite our skepticism on the usefulness of high-throughput technologies in the differential diagnosis between FA and FTC, we anticipate that such methods will provide, in the future, meaningful information for predicting the risk of metastization and the sites of metastization.

For the time being, the histological analysis of exhaustive samples of the tumor capsule is thus necessary to make the differential diagnosis in borderline cases, namely by the identification of vascular invasiveness; if it is not possible to find unequivocal signs of invasiveness, then the designation of 'Follicular tumor of uncertain malignant

potential' advanced by Williams⁴³ is appropriate.^{1,7,24} We realize that the utilization of the term 'uncertain malignant potential' in a pathology report creates a big problem to clinicians. Such a term should be used as rarely as possible, not only because of its 'uncertainty' but also because it is not yet associated with any firmly established management protocol. Such diagnosis should only be made in cases in which one has a thorough evaluation of the whole capsule of the tumor, thus ruling out its utilization in most consultancy cases. The detection of several immunohistochemical and molecular markers has not proved to be diagnostically useful in this setting.^{26,44}

In our institutions, follicular tumors of uncertain malignant potential are treated conservatively. Moreover, we think, like Rosai *et al*,² Rosai,⁷ and other authors^{19,20} that follicular tumors in which capsular invasion is the sole sign of malignancy should also be treated conservatively (see below), but these issues need to be addressed in Multidisciplinary Consensus Conferences to produce management protocols.

Differential Diagnosis between Minimally Invasive FTC and Encapsulated FVPTC

At present, this is the most frequent reason for consultancy in thyroid pathology. A few years ago, it used to be the identification of signs of capsular and/or vascular invasion, whereas nowadays it is 'Whether or not the nuclear characteristics of the neoplastic cells support the diagnosis of PTC.'^{1-3,7,24,45}

The diagnosis of encapsulated FVPTC has probably become the hottest topic in thyroid pathology mainly because of the too-liberal utilization of the PTC nuclear criteria. It should be stressed that this dilemma is restricted to the encapsulated type of FVPTC as the other two types of FVPTCs (such as poorly circumscribed and multinodular/diffuse types) do not usually raise diagnostic problems.^{21,24,45}

We have been stressing, since 2002, the existence of the aforementioned three types of FVPTCs.⁴⁶ These types apparently have different natural histories and display quite different patterns of metastatization.^{15,22,24,34,46,47} Poorly circumscribed/infiltrative FVPTCs metastasize, like conventional PTCs, to regional lymph nodes;^{1-3,44,46} encapsulated FVPTCs tend to give rise, whenever displaying angioinvasiveness, to lung and bone metastases without concurrent nodal metastases;^{47,48} and multinodular/diffuse FVPTCs are characterized by the frequent metastatic involvement of bones and lungs together with lymph nodes.⁴⁹ The peculiar blood-born metastatic pattern of some cases of FVPTCs had been highlighted many years ago by Rosai and colleagues⁵⁰ and Rosai.⁵¹

Besides the low frequency and type of the *BRAF* mutation,^{34,52,53} there are other molecular features

of FVPTCs in general, and of encapsulated FVPTCs in particular, that differ from those of conventional PTCs. Some of those features resemble the molecular alterations that are characteristic of FA and FTC.¹ E-cadherin immunoexpression in encapsulated FVPTCs resembles that of follicular tumors and normal thyroid rather than that of conventional PTCs.^{54,55} The frequency of *RAS* mutations is much higher in FVPTCs than in conventional PTCs,^{22,56,57} thus also contributing to approach the FVPTC to FA and FTC.^{22,51} Similar prevalences of *RAS* mutations were detected in FA (33%), FTC (22%) and FVPTC (25%).²² Roque *et al*⁵⁸ described, in 2001, using conventional cytogenetics, the t(2;3) (q12;p25) in one case of FVPTC, and Wreesmann *et al*⁵⁹ using immunohistochemistry demonstrated, in 2004, overexpression of *PPARγ* in three cases of FVPTCs. Castro *et al*²² detected the presence of *PAX8-PPARγ* rearrangement in 37% of the cases of encapsulated FVPTCs, 33% of FAs and 45% of FTCs, in contrast to its absence in conventional PTCs.^{54,55} The results obtained by Castro *et al*²² in FVPTCs probably reflect the stringent criteria used in the selection of cases of FVPTCs, excluding all tumors with a follicular/papillary growth pattern.

Taken together, the aforementioned data support the conclusion that FVPTCs show many genetic differences from conventional PTCs^{22,59,60} and suggest that at least a subset of FVPTC that apparently corresponds to the encapsulated type of FVPTC shares some of the molecular features of FTC.^{22,57} The same holds true in FTC and FVPTC arising in *struma ovarii* cases.⁶¹

It remains to be seen whether the subset of FVPTC displaying genetic alterations of the FTC type encompasses cases particularly prone to give rise to lung and/or bone metastases. It also remains unclarified whether cases of encapsulated FVPTC lacking signs of vascular invasion metastasize to the lungs or bones regardless of whether having the *PAX8-PPARγ* rearrangement or other genetic alterations. The results obtained so far in our retrospective study of 239 cases of well-differentiated thyroid carcinomas diagnosed in the Hospital S. João and Porto Cancer Institute, from 1978 to 2003, which displayed nodal and/or blood born metastases, has shown that in this group there was not a single case of follicular tumor of uncertain malignant potential or a well-differentiated tumor of uncertain malignant potential, nor any case of minimally invasive, non angioinvasive FTC or encapsulated, noninvasive FVPTC (Magalhães *et al*, unpublished results).

The problem of disclosing the malignant potential of encapsulated, nonangioinvasive FVPTC and, based on such evaluation, to decide the best therapy for such cases is similar to the discussion on the clinical malignancy and therapy selection for minimally invasive FTC with capsular invasion alone (see above). The evidence on record suggests that for both situations, lobectomy or lobectomy plus isthmectomy will be enough.^{18,20,62,63}

Summing up, there are two separate problems in the diagnosis of encapsulated tumors with follicular architecture: to decide whether minor nuclear changes of the PTC type justify a diagnosis of FVPTC and to decide whether a minor degree of capsular penetration justifies a diagnosis of malignancy.⁴³ For the moment and although there is no additional evidence from the molecular pathology side, we agree with Williams and the Chernobyl Study Group⁴³ that it is more appropriate to recognize the difficulty in deciding whether the nuclei are typical than to arbitrarily place well-differentiated encapsulated tumors with a follicular architecture, in which minor nuclear changes are the only indicator of a PTC, in a definite malignant or a definite benign category.⁴³ Like Williams⁴³ and many other authors in Europe,^{1,7,24,26,44,46} we refer to these tumors simply as 'well differentiated' without specifying either follicular or papillary, and use the terms 'well-differentiated tumor of uncertain malignant potential' when there is no definite evidence of invasion, and 'well-differentiated carcinoma, NOS' when invasion is present.^{1,24,64} The term 'well-differentiated tumor of uncertain malignant potential' should be used as rarely as possible and should never be considered as a substitute for adequate sampling of any follicular-patterned thyroid tumor.^{1,65} In our experience, and in that of other groups,^{19,20,62,63} these tumors are adequately treated with a conservative approach (lobectomy or lobectomy plus isthmectomy), but one still needs firmly established management protocols. Although we do not sympathize with the designation 'well-differentiated carcinoma, NOS,' we use it occasionally for two main reasons: some follicular-patterned carcinomas of the thyroid are difficult to characterize because they have intermediate nuclei or different types of nuclei, and the treatment is similar for 'conventional PTC,' 'angioinvasive FTC' and 'angioinvasive, well-differentiated carcinoma, NOS.'

Differential Diagnosis between Widely Invasive FTC and Poorly Differentiated Thyroid Carcinoma

In the WHO book,⁶⁴ PDTC has been recognized as a histopathological entity. The absence of clear diagnostic criteria for PDTC has been recently solved by Volante *et al*¹¹ who described such criteria and advanced an algorithmic diagnostic approach for these tumors.

Despite the aforementioned developments in the diagnosis of PDTCs, it may be difficult to separate them from FTCs with a predominant trabecular/insular/solid growth pattern.^{11,60,61} The same holds true regarding the separation of PDTCs from PTCs predominantly composed of trabecular, insular or solid areas whenever the nuclei of neoplastic cells superficially resemble the PTC nuclei.^{11,64,65}

In both instances (DD with PTC and FTC), the foci of necrosis and high mitotic rate may be used in individual cases as signs suggestive of PDTC but

should never be considered as absolute criteria.^{11,64,65} The evaluation of the nuclear features is crucial whenever one faces the differential diagnosis with PTC; if the nuclei in the 'poorly differentiated' areas are of the PTC type, then we stick to the diagnosis of PTCs with trabecular or solid areas because the prognosis is much better than that of PDTCs.^{11,64}

From a pragmatic standpoint, the separation of PDCs from FTCs only matters when one is dealing with a minimally invasive neoplasm, as widely invasive FTCs carry a prognosis that is similar to that of PDTCs and should be treated just as radically as them.^{11,64,65}

Oncocytic (Hürthle Cell) Variant of FTC

Tumors with oncocytic (Hürthle cell) features may be benign (oncocytic variant of FA) or malignant (oncocytic variant of PTC and oncocytic variant of FTC). Medullary and poorly differentiated carcinomas may also display oncocytic features.^{9,10,66,67}

Hürthle cells are stuffed with the mitochondria with morphological, functional and genetic abnormalities.^{9,10,66,67} The accumulation of the mitochondria may be due to a primary alteration of mitochondrial DNA or of nuclear DNA that encode mitochondrial enzymes.^{9,10,68,69} Regardless of the mechanism(s) involved in the process, a deficient mitochondrial function leads to an increased number of mitochondria through the stimulation of mitochondrial proliferation.^{9,10,68,69}

It has been shown that most of the typical molecular features of conventional PTCs and FTCs are also present in their oncocytic counterpart.^{15,35,52,68,69} For practical purposes, the oncocytic appearance is believed to represent a phenotype that is superimposed to the genotypic and histopathological features of thyroid tumors.

The criteria used in the diagnosis of the oncocytic variants of PTCs and FTCs are those used in the diagnosis of conventional tumors, ie, mainly the nuclear characteristics in PTCs and the signs of capsular and/or vascular invasion in FTCs. The prognosis of patients with the oncocytic variant of PTCs or FTCs is similar to that of patients with the respective conventional carcinomas provided the age of the patients and the staging of the tumors are comparable.^{1,69–71} From a clinical standpoint, the negative aspect of oncocytic carcinomas of the thyroid is their lesser ability to trap iodine,

thus rendering them less responsive to radioactive iodine.^{1,10,72,73}

Although it is now widely accepted that many oncocytic tumors of the thyroid are benign (nodular goiters and FA with oncocytic features), one should keep in mind that the percentage of oncocytic tumors displaying signs of invasiveness is higher than that occurring in tumors without oncocytic features. That is the reason why one should search actively for capsular and vascular invasion, and for the PTC nuclei, whenever dealing with any oncocytic tumor that looks benign at a first glance.^{1–3,7,65}

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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