The overall aim of the present thesis was to progress in the understanding of the pathogenic and diagnostic meaning of some numerical and structural chromosomal abnormalities that are frequently detected in follicular cell derived tumors of the thyroid.

The flow cytometry study of 143 thyroid lesions revealed that the aneuploidy in follicular tumors is strongly associated with a particular morphologic pattern, the so-called fetal type of growth pattern, leading to the individualization of fetal adenomas (FTA) from the DNA content standpoint. Moreover, we found that a high percentage of FTA, as well as follicular thyroid carcinomas (FTC) with a fetal adenoma growth pattern, were within the triploid range.

The results obtained by CGH (and confirmed by FISH) revealed that aneuploidy in FTA and FTC is usually represented by whole chromosome gains. Not all chromosomes are affected in the same way. Despite being frequently triploid, FTA display a highly repetitive pattern characterized by four copies of some chromosomes, three copies of several other chromosomes, and two copies of others. The follicular tumors displaying a distinct DNA content had a usual follicular growth pattern and showed a CGH profile with very few alterations and a trend toward the loss of some chromosomes.

The CGH results suggest that follicular tumorigenesis may follow at least two pathways: one characterized by prominent aneuploidy and numerous gains, in which the tumors display a fetal adenomolike growth pattern, and another pathway accompanied by less obvious aneuploidy or even quasi diploidy and dominant chromosome losses, in which the tumors display a “common follicular architecture.”

To investigate the putative molecular alteration underlying aneuploidization in follicular tumors, the RAS mutational status was investigated in 85 tumors. The results showed that RAS mutations are not a major cause of aneuploidy in thyroid tumors, but there is a strong association between the H-RAS 81C polymorphism and the presence of aneuploidy. Since the H-RAS 81C polymorphism does not alter the RAS protein, the association has to be explained in another way: We advanced that such association could reflect the increased H-RAS mRNA expression found in these tumors, as well as by the preferential expression of the H-RAS p21 isoform, which is the isoform that is able to activate the downstream effectors of RAS.

Besides FTA and FTC, there is a third follicular patterned thyroid lesion, the follicular variant of papillary thyroid carcinoma (FVPTC), which is characterized by consistent diploidy and a disputable pathogenesis. To investigate if this FVPTC, from a molecular...
FIGURE 1. Summary of gains and losses detected by CGH in the fetal adenomas. Gains are represented by lines to the right of the chromosomes, and losses by lines at the left. Each line represents the genetic aberration seen in one tumor.

standpoint, is closer to FTC or to conventional PTC (cPTC), we analyzed a series of 40 FVPTC, 27 FTC and 12 FTA for the mutational status of RAS and B-RAF, as well as for the PAX8-PPAR\(\gamma\) rearrangement. We detected, for the first time, a high percentage of PAX8-PPAR\(\gamma\) rearrangement in FVPTC (37.5%), which is comparable to those found in FTC (45.5%) and in FTA (33.3%). The same holds true regarding the frequency of RAS mutations, 25% in FVPTC, 33.3% in FTC and 33.3% in FTA. The results showed that FVPTC share some molecular feature of FTC, namely, RAS mutations and PAX8-PPAR\(\gamma\) rearrangements, thus ruling out the current idea that FVPTC should be considered as a subgroup of cPTC. Our results also disclosed a third pathway for cancerization of follicular patterned thyroid lesions that, being characterized by consistent diploidy, will be used for comparative purposes with the aforementioned aneuploidy associated pathways.

Comment by Paula Soares

This thesis represents an excellent example of a translational research work in the sense that she starts from the morphology/pathology standpoint and ends with a handful of basic biology questions (that she, hopefully, will address in her postdoctoral work) and new data in the characterization of some thyroid tumors.

In the first work of the thesis, revisiting an old theme in molecular pathology—the DNA content of thyroid tumors—and taking advantage of a criterious classification of the lesions, she performed a flow cytometric study that brings new data and some clarification. She confirmed that aneuploidy can be present in benign and malignant lesions of the thyroid, and verified for the first time that the aneuploid lesions share a particular
morphology, the fetal type growth pattern. Furthermore, in analyzing the DNA index of the lesions, she verifies that the distribution of the DNA content is not random, but concentrates in the triploid range. This observation raises several interesting oncobiology questions: How do thyroid cells become aneuploid? Is it through a first step of tetraploidization and a progressive chromosome loss, or by a crescent acquisition of extra chromosomes? And why do they reach a kind of plateau in the triploid range? Have the tumor cells achieved equilibrium between gains and losses?¹

To go further in these questions, the second work of the thesis characterizes the gain and losses in the aneuploid tumors by CGH and FISH, obtaining data that favors the hypothesis that thyroid cancer cells undergo a first step of tetraploidization followed by a nonrandom process of gains and losses of chromosomes.²

In the third paper of the thesis, Patrícia Castro again returns to an old theme and comes back with new and exciting data. Here, she addresses the role of RAS genes in the aneuploidization of thyroid tumors. It has been long known that RAS mutations are a relatively frequent event in follicular lesions, some authors proposing that a link exists between RAS mutations and aneuploidy. Patricia Castro demonstrates in her thesis that aneuploidy in thyroid lesions is not associated with the presence of RAS mutations, but with a particular genotype in the H-RAS gene. She also verifies that this H-RAS polymorphism is associated with a higher expression of total H-RAS mRNA and with a higher expression of p21 H-RAS mRNA. These results open more questions and experiments in alternative ways of H-RAS activation, other than gene mutation.³

The work in the follicular variant of papillary thyroid carcinoma demonstrated for the first time the presence of PAX8-PPARγ rearrangement in FVPTC (a genetic alteration presumed to be specific to follicular tumors) and strengthens the hypothesis of a kind of hybrid type of thyroid tumor.⁴

REFERENCES


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