Molecular diagnosis of differentiated thyroid cancer. 
Towards the application in clinical practice

Le diagnostic moléculaire du cancer différencié de la thyroïde :
vers une utilisation en pratique clinique

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The preoperative diagnosis of thyroid cancer relies on cytological examination of material collected by fine needle aspiration biopsies (FNAB). Due to the absence of markers, it is difficult, even for experienced cytologists, to discriminate benign from malignant follicular tumors. Consequently, only about 35% of patients undergoing thyroidectomy for cancer or suspicion of cancer, actually present a thyroid cancer. There is need for improvement of the diagnostic procedure to reduce the number of thyroid ablation subsequently proved to be unjustified (about 10,000 per year in France).

The development of high throughput technology of gene expression analyses has opened true possibilities of identification of markers for diagnosis or prognosis of cancer. Since the pioneering work of Huang et al. [1] on the identification of genes differentially expressed in papillary thyroid carcinomas (PTC) versus normal thyroid tissue, numerous studies have identified series of genes exhibiting distinct expression profiles in the different types or subtypes of thyroid tumors [2–11]. Some recent studies have already investigated how gene expression profiling data can be used to predict the type or class of individual tumors particularly with difficult cytological diagnosis such as follicular variant of PTC [12,13] and follicular thyroid carcinomas (FTC) [14–18].

The challenge is now to choose combination(s) of marker genes discriminating benign from malignant thyroid tumors to try to set up molecular diagnostic test(s) in view of an application to FNAB. Because the pangenomic microarray technologies, now readily available, are expensive and cannot be used on large number of samples, we decided to build a dedicated macroarray containing about 200 potentially informative genes that is genes previously found to be differentially expressed in thyroid carcinomas as compared to adenomas and/or normal tissue or between thyroid carcinoma subtypes. To develop this simplified approach, we used a methodology based on the spotting of oligonucleotides on nylon membrane and hybridization of [33P]-labeled cDNA probes. Gene expression profile analyses were performed on samples from the Lyon Thyroid Tumor bank (approved by Inserm and ministry of Research in 2003).

We conducted a two-stage research program named Dépistage du Cancer de la Thyroïde (for DeCanThyr) which consisted in i) the elaboration of gene expression profile-based thyroid tumor classifiers and ii) an evaluation of their capacity to diagnose benign versus malignant tumors on material from FNAB. Stage 1 corresponded to transcript analyses on a series of samples (normal tissue, benign tumors and carcinomas) to identify genes discriminating benign and malignant tumors to generate tumor classifiers utilizing the fewest possible number of genes and exhibiting the highest prediction strength. Stage 2 of the study was devoted to the evaluation of performances of the tumor classifiers on a new set of samples (the “validation set”) corresponding to FNAB (FNAB carried out with the standard technique but on nodules after surgical resection). The molecular diagnosis that is benign versus malignant tumor given by each classifier was compared to the diagnosis given by the pathologist (used as “gold standard”).

From analyses of 56 samples that is follicular adenomas (FA) + normal tissue (NT) (n = 34) and thyroid carcinomas (n = 22), we identified series of 26, 57 and 21 genes capable...
of discriminating FA and NT from follicular thyroid carcinomas (FTC), papillary thyroid carcinomas (PTC) and FTC + PTC, respectively. For validation of macroarray data, transcripts from six genes were assayed by quantitative RT–PCR. Correlation coefficients between expression levels measured by hybridization on macroarray and by RT–PCR varied from 0.93 to 1.0. On a series of 16 genes, we compared our expression data with those generated in six other studies [1,7–9,12,13] based on Affymetrix or other high density microarrays. There was a complete agreement on changes in transcript levels and the amplitude of variations (i.e. fold change) between groups of samples tend to be higher in our study based on radioisotope labeling of probes than in the other studies bringing into play fluorescently-labeled probes.

Data corresponding to the discriminating genes and deriving from the 56 samples (representing the “training set”) were subjected to a weighted voting algorithm [19,20] to generate prediction models capable of assigning a new sample to one of the two classes: benign or malignant. Three prediction models or tumor classifiers were built by considering data from the benign group of samples (FA + NT) versus i) FTC only (the F classifier), ii) PTC only (the P classifier) or iii) FTC + PTC (the common or C classifier). The best F, P and C classifiers (giving the minimal error in cross-validation tests) were composed of nine, nine and 12 genes, respectively. Only two genes belong to the three classifiers. A fourth classifier named global or G classifier was built from the set of 19 genes composing the F, P and C classifiers.

The capacity of the four classifiers to discriminate a benign from a malignant tumor was tested on 26 FNAB samples. The amount of RNA recovered from FNAB was sufficient (greater than or equal to 100 ng) to perform macroarray analyses. The four classifiers yielded the same diagnosis (benign or malignant) in agreement with the diagnosis of the pathologist in 23 out of the 26 FNAB; in the three other cases, the correct diagnosis was given by three of the four classifiers. Thus, the combination of the four classifiers gave the correct diagnosis in 100% of cases with high prediction strength [21].

To have a method fully compatible with an application on a large number of samples, we decided to analyze gene expression profiles by quantitative PCR instead of hybridization on macroarray. We thus proceeded with QPCR measurements of transcript levels of the genes forming the four (above-mentioned) classifiers on a large series of samples (NT, FA, FTC and PTC; n = 140). These gene expression data have been used to generate new forms of tumor classifiers representing the system of reference of the molecular test for further validation steps.

In conclusion, we developed a procedure of molecular diagnosis of benign versus malignant tumors applicable to the material collected by FNAB. The molecular test compiled with a preclinical validation stage; now, its performance must be evaluated on ultrasound-guided FNAB in a large-scale study. With the support of Institut national du cancer (Inca)–PHRC national 2006), we have initiated a prospective study of validation (DeCanThyr II) on a cohort of 800 patients at the Lyon University Hospital (CHU de Lyon).

References

