Journal of Cellular Physiology

Thyroid Tumor Marker Genomics and Proteomics: Diagnostic and Clinical Implications

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Two systems biology concepts, genomics and proteomics, are highlighted in this review. These techniques are implemented to optimize the use of thyroid tumor markers (TTM). Tissue microarray studies can produce genetic maps and proteomics, patterns of protein expression of TTM derived from preoperative biopsies and specimens. For instance, papillary and medullary thyroid cancers harbor *RAS*, *RET*, and *BRAF* genetic mutations. Follicular thyroid cancers harbor translocations and fusions of certain genes (PAX 8 and PPAR-gamma). Proteomic analysis from various tissue sources can provide useful information regarding the overall state of a thyroid cancer cell. Understanding the molecular events related to these genetic and protein alterations can potentially clarify thyroid cancer pathogenesis and guide appropriate molecular targeted therapies. However, despite the realization that these emerging technologies hold great promise, there are still significant obstacles to the routine use of TTM. These include equivocal thyroid nodule tissue morphologic interpretations, inadequate standardization of methods, and monetary costs. Interpretative shortcomings are frequently due to the relative scarcity of cellular material from fine-needle aspiration biopsy (FNAB) specimens. This can be rectified with large needle aspiration biopsy (LNAB) techniques and is exemplified by the favorable performance of galectin-3 determinations on LNAB specimens.

J. Cell. Physiol. 224: 612–619, 2010.

The term "tumor marker" was originally used to indicate a protein in the blood able to signal the presence or suspicion of a tumor. Presently, the utility of tumor markers has been expanded to include the following criteria:

(a) to identify subjects at risk of developing a specific tumor type;
(b) to perform early diagnosis of a primary tumor or its metastasis;
(c) to contribute to histotyping of the tumor;
(d) to contribute to prognosis;
(e) to monitor progression of the tumor, primary or metastatic;
(f) to monitor response to therapy;
(g) to contribute to understanding of tumor pathogenesis;
(h) to suggest diagnostic or therapeutic interventions related to the tumor marker itself or to tumor marker-related molecular events (Van Veelen et al., 2009).

In the specific case of thyroid tumor markers (TTM), the biochemical evaluation of the thyroid cancer patient has advanced from simple serum thyroglobulin (Tg) and calcitonin (CTN) determinations to genomic and proteomic analyses (Giordano, 2008; Krause et al., 2009). For several decades, many controversies existed regarding the management of thyroid nodules highlighting the need for strong and reliable TTM that would be useful in routine clinical practice. This article will extend the findings of our previous reviews on TTM to focus on these two systems biology techniques (Carpi et al., 2006a; Mechanick and Carpi, 2008).

Research Techniques

Genomics

These techniques represent an evolution of traditional cytogenetics and suggest important associations between molecular mechanisms of carcinogenesis and TTM (Giordano, 2008). The types of thyroid cancers, their respective TTM, and applicable molecular mechanisms are provided in Table 1. The chromosomal and genome-wide techniques used for these studies are principally derived from microarray technologies (Brentani et al., 2005; Mockler et al., 2005; Carter, 2007), which include numerous high-density platforms: robotic deposition ("spotting") of DNA molecules, and short oligonucleotides synthesized in situ (Stoughton, 2005; Lubitz et al., 2006; Mandruzzato, 2007).

RNA is extracted, purified, amplified, labeled, and hybridized for gene expression analysis (Lubitz et al., 2006). Results are expressed as maps of genes characterized by different degrees of activation (Lubitz et al., 2006; Wiseman et al., 2008) or as groups of specific molecular markers characterized by different degrees of expression (i.e., under- or over-expression) (Liang et al., 2009). In the latter case, the degree of expression is evaluated by the immunohistochemistry of each protein and assessed by the proportion of the immunopositive cells and the staining intensity (Liang et al., 2009). According to the above-mentioned genetic studies, tissue microarray studies show different genetic maps or protein expression of TTM, derived from preoperative biopsies and operative specimens (Finley et al., 2004; Lubitz et al., 2006; Eszlinger et al., 2007; Wiseman

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Received 24 March 2010; Accepted 29 March 2010

Published online in Wiley InterScience (www.interscience.wiley.com.), 21 May 2010.
DOI: 10.1002/jcp.22187

TABLE 1. Molecular markers of thyroid tumors derived from genomic and proteomic methods (modified from J.I. Mechanick and A. Carpi, 2008)

(a) Papillary thyroid cancer (PTC)
Pathogenesis
Abnormal mitogenic signaling to the nucleus via the RET/RAS/BRAF–MAPK pathway
Epigenetic inactivation (abnormal promoter methylation) of tumor suppressor, *RASSF1A*, and *TSH-R* gene
Galectin-3 promotes K-Ras signaling to Raf and PI3K, which affects apoptosis
CD44v6 encodes membrane glycoproteins affecting tumors
Metallothionein 1G oncosuppression
Molecular markers
RET/PTC1 > *RET/PTC3*, *TRK* rearrangements, *BRAF* (T1799A, V600E), *RAS* activating mutations, ↑ c-met, EGF-R expression, E-cadherin downregulation, galectin-3, CD44v6, $P27^{KIP1}$, cyclin D1, p53 inactivation, β-catenin, periostin gene expression, H4-PTEN mutation, SAGE: PTPRC + LIMD2 in LN, MDM4 alterations, IL 13, CD 10, trisomy 17, telomerase
(b) Follicular variant of PTC
Pathogenesis
There is a greater tendency toward encapsulation compared with conventional PTC leading toward more hematogenous spread and pulmonary metastasis
Molecular markers
BRAF (A1802G, K601E) mutations, E-cadherin, *RAS* mutations, PPARγ overexpression, *RET/PTC3* rearrangement, *PAX8–PPARγ* rearrangement, 6-gene model (*SYNGR2*, *LSM7*, *KIT*, *Hs.296031*, *c21orf4*, and *Hs.24183*); molecular expression signature (CSNK1G 2, HLA-DQB1, MT1X, RAB 23)
(c) Follicular thyroid cancer
Pathogenesis
Vascular and capsular invasion
Molecular markers
Aneuploidy, loss of heterozygosity, *RAS* mutations, *PAX8–PPARγ* rearrangement, 3-gene model (*cyclin D2*, *PCSK2*, *PLAB*), *hTERT* gene expression, *HMGA2* upregulation, reduced expression of *FZD-1* gene, QRT protein, telomerase
(d) Hurthle cell thyroid cancer
Pathogenesis
Large deletions and point mutations in mtDNA inhibits apoptosis, increases HIF-1, and increases angiogenesis
Subsequent *RET/PTC* rearrangements and/or *BRAF* mutations induce malignant transformation and loss of NIS function
Molecular markers
Overexpression of *PRC*, *NRC1*, *TFAM*, *GRIM-19* downregulation
(e) Medullary thyroid cancer
Pathogenesis
Arises from parafollicular CTN producing C-cells
Tyrosine kinase RET signaling cascade
Molecular markers
CTN, CEA, *RET* protooncogene, *pRb*, *p53*, *PTEN*, *p27Kip1*, *p18-INK4c* mutations, loss of heterozygosity in VHL tumor suppressor locus, reduced MMP-2/TIMP-2 expression
(f) Anaplastic thyroid cancer
Pathogenesis
Undifferentiated, multistep carcinogenesis involving p53
E_2 is proapoptotic in ATC (but growth promoting in PTC)
Overexpression of gene cluster
Deregulation of miRNAs
Thyroid cancer derived from fetal thyroid (stem) cells
Anaplastic transformation
Molecular markers
Panel: TG, Bcl-2, MIB-1, apo E overexpression, RXRγ expression, PLK1 kinase expression, miRNA overexpression, onfFN overexpression, E-cadherin, p53, β-catenin, TOPO-II, VEGF

et al., 2008). The principal data from these studies are summarized as follows (Fig. 1).

Papillary thyroid cancer (PTC) is characterized by certain genetic mutations (*RAS*, *RET/PTC*, and *BRAF*). *BRAF* mutations represent the most specific PTC mutations despite a low accuracy (about 50%). These mutations are associated with loss of radioiodine (RAI) avidity, increased nodal metastases, poor survival, and decreased expression of thyroid-specific genes (like the thyroid-stimulating hormone [TSH] receptor and thyroperoxidase) typical of differentiated PTC. Either *BRAF* or *RET* activation can cause PTC and the activation of both seems to be associated with higher degrees of aggressiveness (Carpi et al., 2006a; Mechanick and Carpi, 2008; Kato and Fahey, 2009; Eszlinger and Paschke, 2010).

Follicular thyroid cancer (FTC) is characterized by translocations and fusions of certain genes (e.g., *PAX8* and *PPAR*-gamma) and expression of specific proteins. Genomic copy gain and amplification of *PIC 3CA* as well as *RAS* mutations are commonly found in FTC (Eszlinger and Paschke, 2010). Almost all of these genetical alterations can activate the mitogen-activated protein (MAP) kinase and P13K/AKT pathways in thyroid cancer. These pathways are also activated by aberrant methylation of important tumor suppressor and thyroid-specific genes. Therefore, these genetic markers are becoming useful to develop therapeutic strategies targeting the MAPK and P13K/AKT pathways (Schlumberger et al., 2009).

Medullary thyroid cancer (MTC) is also characterized by RET mutations and activation of the tyrosine kinase signaling cascade. Also in MTC, these complex and sequential molecular events can be used for molecular targeted therapy (Schlumberger et al., 2009; Van Veelen et al., 2009). In MTC, important results in diagnostic imaging and therapy are obtained following the studies on traditional serum TTM, such as CTN and carcinoembryonic antigen (CEA), and novel markers like somatostatin receptor (SSTR), gastrin/cholecystokin B receptor (CCKBR), and glial family of receptor alfa (GFRα)-4 (Van Veelen et al., 2009).

Anaplastic thyroid carcinomas (ATC) harbor frequent point mutations in the p53 tumor suppressor gene. The disruption of the p53 protective effects seemed to be relevant in the progression of thyroid neoplasms to an aggressive, undifferentiated phenotype. Codons 273 and 248 are hot spots for the p53 mutation in ATC (Ito et al., 1992).

Proteomic studies

The term "proteomics" describes the global analysis of the proteome, which is composed of the total collection of all proteins expressed at the given time point and physiological state in a specific biological entity (Brentani et al., 2005). The hypothesis is that a specific pattern of protein synthesis reflects a specific thyroid cancer histotype. The "transcriptome" is the complete set of RNA transcripts produced by the genome.

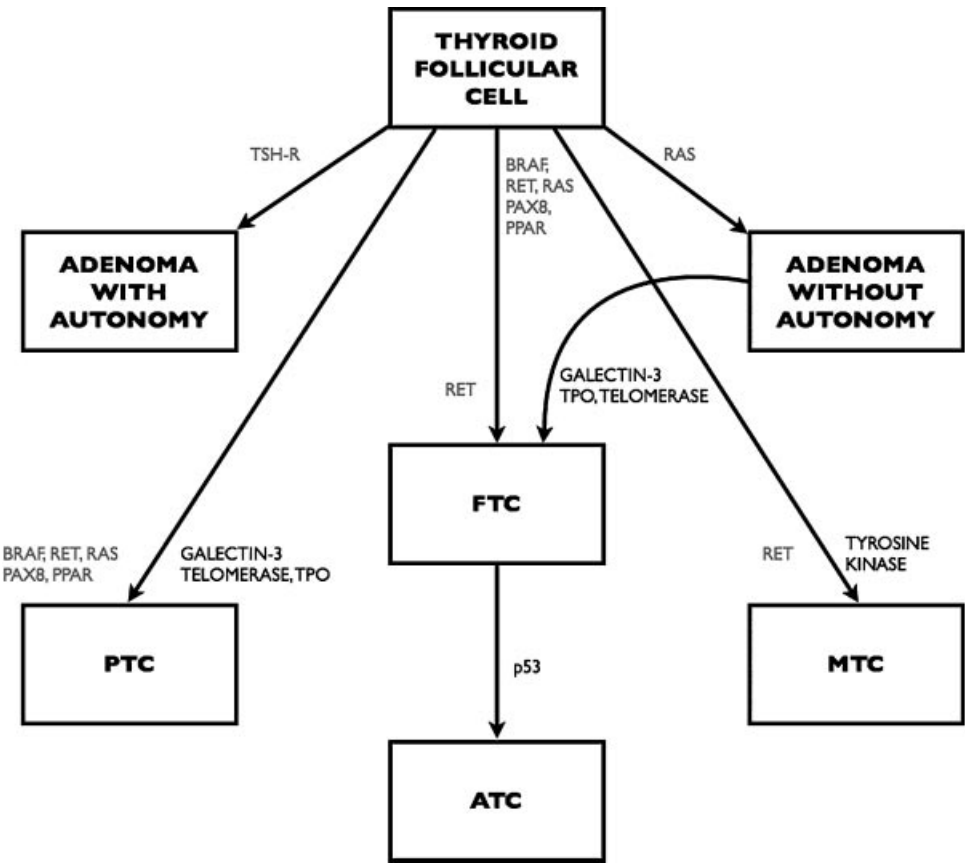

Fig. 1. Thyroid tumor markers derived from genomic and proteomic methods. The gray markers are derived from genomic methods; the black markers are derived from proteomic methods. See Table 1 for a more complete list of markers.

Genomic, transcriptomic, and proteomic studies have significantly advanced our understanding in thyroid carcinogenesis (Giordano, 2008; Krause et al., 2009) because accumulated genomic alterations are expressed into the transcriptome and then translated to the proteome with numerous modifications. Together, these processes reflect the transformed state of malignant tumor cells. Proteins can be identified, extracted, and quantified with different techniques on serum, thyroid tissue, or on cell lines (Lubitz et al., 2006; Arcinas et al., 2009; Krause et al., 2009). These techniques are relatively new and are briefly summarized in Table 2 (Brown et al., 2006; Moretz et al., 2008; Netea-Maier et al., 2008; Wang et al., 2006).

The first technique is the two-dimensional gel electrophoresis (2D-GE). In this technique, proteins are first separated according to their net charge by isoelectric focusing followed by the separation according to their molecular masses by polyacrylamide gel electrophoresis (PAGE). Proteins of interest can be excised from 2D gels and then analyzed by mass spectrometry (Krause et al., 2009). By the surface enhanced laser description/ionization (SELDI)-TOF technique, samples can be directly profiled by mass spectrometry without protein

TABLE 2. Proteomics techniques and thyroid tumor markers (modified from K. Krause et al., 2009)

Study source	Main technique	Principal finding
Thyroid tissue (Brown LM et al., 2006)	2D gel electrophoresis	31 proteins differentially expressed in PTC and NT (S100 A6 protein 6.5-fold higher in PTC)
Thyroid tissue (Netea-Maier RT et al., 2008)	2D gel electrophoresis	FTC > FA, histone H2B, cytokeratin 7, FTC < FA, cytokeratin 8 78-kDa, glucose-regulated protein, calreticulin, annexin A3, β-actin
Serum (Moretz WH et al., 2008)	SELDI-TOF-MS	Serum expression patterns allowing discrimination between PTC vs. benign nodules
Serum (Wang JX et al., 2006)	SELDI-TOF-MS	Serum expression pattern allowing discrimination between: (1) PTC vs. healthy; (2) PTC vs. benign nodules; (3) different stages of PTC; (4) different pathological types of thyroid cancer
Cell culture (Arcinas A et al., 2009)	Glycoprotein denaturation, digestion, solid-phase extraction, and ESI-MS/MS analysis identification	An average of 150 glycoproteins per cancer cell line (papillary, follicular, Hurthle, anaplastic) were identified. Some glycoproteins are specific of differentiated or anaplastic thyroid tumors

separation on 2D gels (Wright, 2002; Brozkova et al., 2008). Protein lysates can be immobilized on the solid surface of a protein biochip and then analyzed (Wright, 2002; Brozkova et al., 2008). A further technique, phosphoproteomics, analyzes phosphorylated proteins, which provide information about specific cellular metabolic states, such as proliferation and apoptosis (Krause et al., 2009).

More recently protein profiles of human thyroid cancer have been obtained from specific tumor cell lines. In these studies, glycoproteins undergo specific denaturation processes, digestion, solid-phase extraction, and electrospray ionization tandem mass spectrometry ESI-MS/MS analysis identification (Arcinas et al., 2009). Preliminary results show that proteomics vary with different thyroid cancer histotypes. However, Van Staveren et al. (1992) demonstrated that thyroid cell lines do not manifest the conserved properties of in vivo tumors. Furthermore, their results indicate that these cell lines were derived from differentiated and undifferentiated tumor types but have evolved in vitro phenotypes and gene expression profiles similar to undifferentiated tumors. These phenotypic changes include the absence of expression of most thyrocyte-specific genes, the non-responsiveness to thyrotropin, as well as their large number of chromosomal abnormalities. Thus, thyroid cell lines retain some properties of the cells of origin, for example, genetics, epigenetics, and gene expression, but they show clear differences in these properties compared to in vivo tumors. These results must be incorporated when interpreting thyroid cell line studies.

Obstacles to Translating Genomic and Proteomic Methodologies

Genomic, transcriptomic, and proteomic analyses of preoperative FNAB specimens have the potential to improve preoperative diagnosis of benign and malignant thyroid nodules (Fig. 1). This could optimize the surgical selection process and therefore result in more effective treatment (Giordano, 2008). Unfortunately, traditional FNAB performance and interpretation show important limitations (Carpi et al., 2008) of which the main one is the discrimination of the follicular patterned lesions (Carpi et al., 2005, 2006b; Bartolazzi et al., 2008).

General obstacles

The task of preoperatively discriminating hyperplastic and neoplastic (follicular adenoma and carcinoma with follicular structure) lesions is problematic. A recent study of Hirokawa et al. (2002) analyzed the factors contributing to observer variation among eight pathologists from different institutions who reviewed the same postoperative studies of 21 encapsulated follicular lesions. The frequency of diagnosis of benign adenomatous goiter among Japanese pathologists (31%) was considerably higher than that among American pathologists (6%). In contrast, the frequency of diagnosis of PTC among American pathologists (25%) was considerably higher than that among Japanese pathologists (4%). The analysis revealed three main factors affecting interobserver variations: (1) interpretation of the significance of microfollicles intimately related to capillaries within the tumor capsule; (2) evaluation of nuclear clearing indicative of PTC, and (3) absence of clear morphologic criteria for separation of adenomatous goiter and follicular adenoma (Hirokawa et al., 2002). Considering these findings, it is not surprising that the postoperative incidence of follicular neoplasms (follicular adenoma plus follicular carcinoma) in various series of thyroid nodules with follicular structure by FNAB varies widely from 6% to 80% (Carpi et al., 2005). Moreover, the relative frequencies of the cancer histotypes observed postoperatively in these nodules are also very different (Segev et al., 2003; Carpi et al., 2005; Deveci et al., 2006). Overall, hyperplasia, adenoma, and carcinoma each occurs in about one-third of cases (Segev et al., 2003; Hooft et al., 2004; Deveci et al., 2006) with follicular variant of PTC being the most frequent postoperative finding (Segev et al., 2003; Deveci et al., 2006). These data underscore the importance of follicular architecture in the diagnosis of these types of nodules.

Specific obstacles

The techniques for transcriptomic and proteomic analyses are expensive, not yet familiar to routine laboratories, and without adequate standardization (Eszlinger et al., 2007; Krause et al., 2009). Furthermore, the correlation between transcriptomic and proteomic results in the same type of nodular disease is poor or absent (Krause et al., 2009). The antibodies available for many molecular markers diagnostic techniques demonstrate considerable variability in sensitivity and specificity (Bartolazzi et al., 2008; Serra and Asa, 2008). No doubt, the scarcity of many FNAB specimens will tax any assay (Segev et al., 2003). Some markers, like galectin-3, require an optimal substrate, such as formalin-fixed and paraffin-embedded cell-block preparations, rather than conventional FNAB smears (Bartolazzi et al., 2008). However, many thyroid aspirates have low cellularity and are not suitable for cell-block immunochemistry (Mills et al., 2005). The amount and the quality of RNA available from FNAB are also limiting. Therefore, an assay based on a limited number of differentiating genes, identified by sophisticated algorithms in comparative studies, may be applicable to FNAB specimens (Jarzab et al., 2005; Weber et al., 2005). Nevertheless, in contrast to the analysis of tumor-specific mutations, the approach of quantitatively measuring RNA markers is more susceptible to present potential limitations using FNAB. In fact, limited and variable numbers of follicular cells are obtained in each biopsy. Some authors point out the potential contamination by other cell types such as macrophages (Matesa et al., 2007) or activated lymphocytes in patients with lymphocytic thyroiditis. This may be addressed with a correction for mRNA yield (e.g., by measuring a housekeeping gene like β-actin) and thyroid specificity of mRNA extracted from a FNA sample (e.g., by measuring a thyroid-specific gene like Tg) (Eszlinger et al., 2007) as well as a correction for Tg in the proteome. Moreover, quantitative molecular methods lack a crucial element in thyroid pathology: the morphology of the specimen. This shortcoming exacerbates problems of sensitivity and specificity since only a small fraction of the FNAB sample consists of thyroid epithelial cells (Segev et al., 2003). Chen et al. (2008) reported that for microRNA analysis, paraffin sections were a better substrate than FNAB samples. In a multicenter study, Liang et al. (2009) described in a comparative analysis of protein expression in differentiated thyroid tumors that (1) a mean of five hematoxylin–eosin slides were evaluated per patients, (2) tissue microarrays were constructed using 1 mm diameter tumor cores taken from appropriate areas of the formalin-fixed paraffin-embedded tissue blocks, and (3) serial sections 4 mm thick were cut from the tissue microarray blocks. Siddiqui et al. (2001) demonstrated that FNAB slides older than 3 years were inadequate for evaluation of telomerase reverse transcriptase gene expression. Samija et al. (2008) described that 13%, in the case of one puncture, or 21%, in the case of two punctures, of the FNAB samples were inadequate for the reverse transcriptase-polymerase chain reaction (RT-PCR) expression analysis of glyceraldehyde-3-phosphate dehydrogenase and thyroglobulin.

The use of the LNAB technique has been reported to solve the problems related to the paucity of the FNAB specimens and the lack of its morphology (Carpi et al., 1996, 2010, 2006b; Bartolazzi et al., 2008). In fact, LNAB provides a specimen

adequate for histological examination and a tissue substrate more abundant and adequate for TTM detection than FNAB (Gasbarri et al., 2004; Carpi et al., 2007). The residual biological material available in 150 FNAB-derived cell blocks and 200 LNAB-derived histological blocks after galectin-3 determination has been compared. Only 1–2 sections were still available for further study in only 10% of the FNAB cell blocks, whereas >5 sections could be obtained from 97% of the LNAB blocks (Carpi et al., 2007). As a result of the increased tissue obtained with LNAB for galectin-3 immunodetection, there was more likelihood of having adequate formalin-fixed cell blocks rather than isolated cells, less non-specific galectin-3 immunoreactivity, and hence a lower false-positive rate (Bartolazzi et al., 2008). Furthermore, LNAB-derived cell-block preparations allow also a comparative immunocytochemical assessment, on the same cytological slides, of different antigens associated with thyroid cancer (Bartolazzi et al., 2008).

Since one or more molecular markers can be considered for decision tree analysis, and since FNAB can be an insufficient substrate, LNAB emerges as a better source of substrate for the pathologist. Furthermore, if we consider that LNAB can be obtained from many nodules with inadequate FNAB (Carpi et al., 1998), there will be more nodular tissue to provide a histopathological diagnosis and allow TTM testing to establish a diagnosis of benign nodule (Carpi et al., 2010).

Currently Used and Validated TTM

Table 3 shows the currently used and clinical validated TTM in the various cancer histotypes. They are grouped according to the site of production or detection and their clinical use.

Preoperative TTM

Plasma CTN. Plasma CTN level is a specific biomarker for the diagnosis of MTC and C-cell hyperplasia (CCH). The CTN cut-off values are less than 10 or 20 pg/ml according to the method (Van Veelen et al., 2009). However, occasionally, some cases of MTC do not secrete calcitonin; age, obesity, and cigarettes smoking can increase plasma CTN (Hahm et al., 2001; Elisei et al., 2004; Karanikas et al., 2004; Papi et al., 2006; Costante et al., 2007; D'Herbomez et al., 2007). Germ line mutation analyses can detect hereditary MTC or a risk of developing MTC, which is nearly 100% (Van Veelen et al., 2009). A calcium-pentagastrin stimulation test can identify patients with CCH or MTC but decreased pentagastrin availability and increased genetic testing availability has reduced the need for this diagnostic maneuver.

Plasma calcitonin determinations to screen for MTC during the routine evaluation of a thyroid nodule in low-risk patients are controversial and not recommended by recent clinical practice guidelines (Van Veelen et al., 2009). This is due to the very rare occurrence of MTC among all thyroid nodules investigated (American Thyroid Association, 2009).

Tissue and serum Tg

Thyroglobulin detection can be performed in biopsy material (solid or cystic) to assess the thyroid origin of an extrathyroid mass in a patient with, or suspected to have, thyroid cancer. In the case of a thyroid nodule in the neck, serum Tg determination is indicated only for detecting cancer recurrence or metastasis after total or near-total thyroidectomy with postoperative RAI ablation (American Thyroid Association, 2009).

[Recently, in a prospective pilot study including 71 patients with multiple thyroid nodules and 13 patients with solitary thyroid nodule, serum galectin-3 determinations detected 87% of macro-papillary carcinomas and 67% of micro-papillary carcinomas. The serum galectin-3 test was more sensitive when multiple thyroid nodules were present, detecting 74% of PTC in the multiple nodule group and only 11% of PTC in the solitary nodule group (Saussez et al., 2008)]

Thyroid nodule TTM

The use of validated serum TTM to complement FNAB cytology can enhance the evaluation of follicular lesions where an accurate diagnosis can be elusive due to indeterminate cytological findings. Fifty potential TTM have been analyzed and five of these (thyroid peroxidase [TPO], telomerase, galectin-3, RET-PTC, and p53) were selected due to relatively high accuracy for detecting thyroid cancer in nodules with indeterminate FNAB findings (Haugen et al., 2002).

The expression of many individual genes (Haugen et al., 2002; Lubitz et al., 2006) and seven microRNA expression profiles (Karger et al., 2006; Nikiforova et al., 2008) have been analyzed on FNAB specimens. Recently, TPO and galectin-3 immunostaining in differentiated thyroid carcinoma have been correlated to the biological aggressiveness of the carcinoma (Savin et al., 2008). Galectin-3 has been among the few TTM subjected to wide multicenter studies (Bartolazzi et al., 2001, 2008). Galectin-3 is a member of the β-galactoside-binding family of lectins and has been implicated in various biological processes such as cell adhesion, cell recognition, proliferation, differentiation, immunomodulation, organization of the extracellular matrix, and metastasis (Saggiorato et al., 2001; Inohara and Raz, 1995).

Cell surface galectin-3 mediates cell–cell and cell–matrix interaction, nuclear gal-3 mediates pre-mRNA splicing, and cytoplasmatic galectin-3 mediates the antiapoptotic effects and tumor progression (Inohara and Raz, 1995; Akahani et al., 1997). Alteration of galectin-3 expression has been reported in a variety of tumors, including colon, breast, stomach, brain, and thyroid (Irimura et al., 1991; Lotan et al., 1994; Cvejic et al., 1998; Kim et al., 2006).

In thyroid malignancy, tissue galectin-3 is over-expressed while it is not usually expressed in normal thyroid tissue. Galectin-3 is therefore considered as a marker of early malignant transformation. Nearly all PTC, and most-to-all (82–100%) FTC, are galectin-3 immunopositive (Haugen et al., 2002; Serra and Asa, 2008). Some non-thyroid cells (squamous cells, fibroblast, and inflammatory cells) can also express galectin-3 and therefore account for a small false-positive rate.

There are six exemplary studies on preoperative detection of galectin-3 in thyroid tissue (Collet et al., 2005; Saggiorato et al., 2005; Carpi et al., 2006b; Kim et al., 2006; Matesa et al., 2007; Bartolazzi et al., 2008): five from a single institution and

TABLE 3. Thyroid tumor markers used or validated for clinical practice

Thyroid tumor marker	Determination site	Determination time	Cancer type	Clinical use
Thyroglobulin	Blood	Postoperative	Differentiated	Indicator of recurrence
Thyroglobulin	Lymph node tissue	Preoperative	Differentiated	Diagnosis of metastases
Calcitonin	Blood	Postoperative	Medullary	Diagnosis
Germ line RET mutation	Blood	Postoperative	Medullary	Prevention, diagnosis
Galectin-3	Nodular tissue	Preoperative	Differentiated	Increases diagnostic specificity
BRAF	Nodular tissue	Preoperative	Papillary	Surgical extension, prognosis

one (Bartolazzi et al., 2008) multicenter study (Table 2). Different techniques were used in these studies with the most common being FNAB formalin-fixed paraffin-embedded cell blocks and immunochemistry. The size of the series with indeterminate follicular nodules at FNAB varied from 34 to 465 cases. The specificity for galectin-3 to diagnose thyroid nodules with indeterminate follicular cytology by FNAB varied in the studies. The highest value (97.2%) was from a single institution study using LNAB substrate and biotin-free immunoperoxidase staining (Saggiorato et al., 2005; Carpi et al., 2006b). The lowest value (74.8%) used the RT-PCR method, which was associated with false-positive results due to macrophages and Hürthle cells (Matesa et al., 2007).

Another important issue is the galectin-3 expression in preoperative percutaneous biopsy specimens from encapsulated follicular tumors harboring minimal morphologic features of malignancy, such as follicular adenoma, follicular tumors of uncertain malignant potential (FT-UMP), and minimally invasive FTC. Follicular adenomas are benign encapsulated tumors that exhibit a variety of morphologic patterns and in some cases cannot be easily differentiated from minimally invasive FTC and the follicular variant of PTC (Rosai, 2004). In Table 2, the proportion of the diagnoses of follicular adenoma among all the postoperative diagnoses varied from 15% to 40%. In one study, galectin-3 expression was observed in three (50%) of six adenomas studied (Carpi et al., 2006b). A large prospective multicenter Italian study on 465 nodules showed 19 (11%) galectin-3-positive follicular adenomas among 176 tested (Bartolazzi et al., 2008). Matesa et al. (2007) reported galectin-3 expression using RT-PCR in preoperative FNAB from 5 of 17 (29%) follicular adenomas. Saggiorato et al. (2005) described a lower proportion (6%) of adenomas that were positive for galectin-3 on preoperative cytology. However, they defined "positive" galectin-3 immunostaining when it occurred in at least 10% of neoplastic cells (Saggiorato et al., 2005). This contrasts with the above studies in which a smaller amount of galectin-3 in follicular thyroid cells was considered as a positive finding. According to the hypothesis that galectin-3 expression is a marker of the thyroid cell transformation toward malignancy (Bartolazzi, 2000; Bartolazzi et al., 2001), the adenomas with focally positive galectin-3 may in fact be tumors undergoing malignant transformation (Bartolazzi, 2000; Bartolazzi et al., 2001).

Minimally invasive FTC is a grossly encapsulated tumor. The pattern of growth usually resembles that of an adenoma of embryonal, fetal, or atypical type. It has been suggested that some of these cases represent malignant transformation of an adenoma (Rosai, 2004). Since blood vessel invasion is almost never evident grossly, diagnosis relies on minimal, but entire thickness, infiltration of the capsule (Saggiorato et al., 2001). In a retrospective series, 16 of 17 preoperative FNAB cell blocks from this histologically verified tumor type expressed galectin-3 (Saggiorato et al., 2001).

FT-UMP are capsulated tumors with questionable capsular invasion and without PTC-type nuclear changes (Rosai, 2004; Papotti et al., 2005). In the few series reported all (Kim et al., 2006), or almost all (Savin et al., 2008), of the FT-UMP examined expressed galectin-3 in preoperative FNAB cell blocks (Kim et al., 2006) or postoperative tissue sections (Bartolazzi et al., 2001).

The data from these three particular follicular tumor types suggest that galectin-3 increases its expression proportionally to the morphological probability or evidence of malignancy. It has been recommended that in the nodules with indeterminate follicular and negative galectin-3 finding at FNAB, cytokeratin CK19 or Hector Battifora mesothelial-1 (HBME)-1 are detected according to the oncocytic or non-oncocytic FNAB cytology picture (Saggiorato et al., 2005). Carpi et al. (1996) reported that LNAB histology reduced the number of these nodules indeterminate at FNAB and this diagnosis is further improved by the detection of galectin-3 on LNAB (Carpi et al., 2006b).

An FNAB finding of indeterminate follicular nodule is followed by a significant proportion of PTC results at postoperative examination (Carpi et al., 2005). *BRAF* mutation testing of FNAB is specific but not sufficiently sensitive for accurate diagnosis of PTC. A recent investigation (Xing et al., 2009) on the T1799A *BRAF* mutation in thyroid FNAB specimens of 190 patients before thyroidectomy for PTC showed that this mutation strongly predicted extrathyroidal extension, thyroid capsular invasion, and lymph node metastases. Therefore, this test can be used for planning surgery and overall management strategies in patients with PTC. However, *BRAF* mutation analysis cannot be performed on all palpable thyroid nodules due to resource availability and cost. This raises the question of whether it be tested following all definite or suspected FNAB diagnosis of PTC. In this case, the test could be performed on the initial aspiration biopsy specimen or on a subsequent evaluation. Of note, the initial performance of LNAB permits analysis of archival tissue blocks on which TTM and molecular studies can be performed (Carpi et al., 2006b; Bartolazzi et al., 2008). Further clinical trials are needed to determine the most cost-effective protocol to utilize this diagnostic tool.

Postoperative TTM

Serum Tg. Measurement of the serum Tg level is used to monitor patients for residual or recurrent thyroid cancer. One gram of normal thyroid tissue or well-differentiated tumor releases about 0.5 mg/L of Tg when serum TSH is suppressed. The assays used to measure serum Tg must be sensitive and without any interference from anti-Tg autoantibodies (Iervasi et al., 2006; American Thyroid Association, 2009).

TSH (rhTSH)-stimulated Tg measurements are usually more sensitive than non-stimulated Tg measurements obtained during thyroid hormone suppression of TSH. However, a Tg assay with a functional sensitivity of 0.1 mg/L can reduce the need to perform TSH-stimulated Tg measurement. Patients operated for differentiated thyroid cancer and then found to be free of thyroid remnant tumor are expected to have Tg levels less than 0.1 mg/L with suppression therapy. When periodic measurement shows progressively increasing serum Tg levels or anti-Tg levels (Aras et al., 2007; Coelho et al., 2008; Kim et al., 2008; Pedrazzini et al., 2009), cancer metastases or relapse must be sought using anatomical and/or functional imaging techniques. Neck ultrasounds and RAI whole body scans (WBS) are performed; if they are negative, 18-FDG-PET scanning is recommended. However, there are aggressive or poorly differentiated thyroid cancers that may progress despite suspicious serum Tg or anti-Tg levels so when clinically indicated, these aforementioned imaging studies are still indicated.

CTN and CEA. Patients with persistently elevated or rising plasma CTN levels after initial surgery for MTC should be periodically evaluated with imaging techniques to define the extent of any local disease. Many imaging techniques are available for this purpose. Some of these techniques are based on detection of biomarkers which are taken up by MTC cells or which bind to MTC-specific receptors (Van Veelen et al., 2009). Moreover, elevated plasma levels of CEA (>5–10 ng/ml) are associated with tumor size, number of lymph node metastases, MTC recurrence, and prognosis (Wells et al., 1978; Rougier et al., 1983; Machens et al., 2007).

Conclusions

Laboratory and clinical research provide a large number of biomarkers useful or potentially useful for the management of

patients with a thyroid tumor. The application settings for the use of TTM vary from preoperative selection of thyroid nodules for surgery to postoperative follow-up including monitoring of metastatic disease. The latter application suffers from the lack of evidence-based recommendations and consists primarily of consensus statements that vary over time. However, Tg remains a consistent and essential tool. On the other hand, the former application is more grounded in data. Preoperative screening algorithms affect a larger number of patients (those with thyroid nodules compared to those with thyroid cancer) and can effectively reduce surgical excisions of benign nodules. Unfortunately, many of these techniques are too complicated or costly to be routinely used in clinical laboratories or are as yet poorly standardized. Nevertheless, the TTM determinations on preoperative percutaneous biopsy specimens are becoming more feasible. For example, the use of LNAB, which yields more tissue than FNAB, and the recently standardized galectin-3 immunodetection method, which confers accurate information regarding cancer risk, represents two emergent technologies that can optimize the preoperative selection of thyroid nodules.

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