The American Journal of Surgery (2014) 207, 596-601

The American Journal of Surgery®

Clinical Science

Evaluation of genetic biomarkers for distinguishing benign from malignant thyroid neoplasms

Sapna Nagar, M.D.[a,*], Samreen Ahmed, M.P.H.[b], Claire Peeples, M.D.[a], Nichole Urban, M.D.[a], Judy Boura, M.S.[c], Bryan Thibodeau, Ph.D.[b], Jan Akervall, M.D., Ph.D.[a,b], George Wilson, Ph.D.[b,d], Graham Long, M.D.[a], Peter Czako, M.D.[a]

[a]Department of Surgery Beaumont Health System, 3811 West 13 Mile Road, 104-RI, Royal Oak, MI 48073, USA; [b]Beaumont BioBank, [c]Department of Biostatistics, and [d]Department of Radiation Oncology, Beaumont Health System, Royal Oak, MI, USA

KEYWORDS:
Follicular thyroid cancer;
Fine-needle aspiration;
Gene expression;
Real-time polymerase chain reaction

Abstract

BACKGROUND: Fine-needle aspiration (FNA) aids in the diagnosis of thyroid nodules. The expression of previously implicated genes was examined to potentially discriminate between benign and malignant thyroid samples.

METHODS: Patients included for study had cytology demonstrating follicular cells of undetermined significance, atypical cells of undetermined significance, follicular neoplasm, or suspicion of malignancy with one of the following postoperative diagnoses: follicular thyroid adenomas, follicular thyroid carcinomas, or follicular variant of papillary thyroid carcinomas (FV-PTCs). FNA and tumor expression of human telomerase reverse transcriptase (hTERT), high-mobility group A2 (*HMGA2*), and trefoil factor 3/3-galactoside-binding lectin (T/G ratio) were analyzed.

RESULTS: T/G ratios were not significantly different in the malignant and benign groups. *HMGA2* was overexpressed in carcinoma states; however, only FV-PTCs were significant ($P = .006$). Tumor *hTERT* expression was detected in 25% of follicular thyroid carcinomas, whereas 5% of FV-PTCs and 10% of follicular thyroid adenomas had expression. FNA aspirates showed similar results.

CONCLUSIONS: Although *HMGA2* and *hTERT* showed differential expression, they did not consistently differentiate benign from malignant. Further study based on global gene expression is needed to identify markers that could serve as a diagnostic tool.

Thyroid nodules are common, affecting as many as 4% to 7% of the adult population.[1] Of all thyroid nodules detected clinically, only about 5% to 15% are found to contain malignancy.[2] Although thyroid carcinoma accounts for only 1% of all new malignancies, most of these (approximately 90% to 94%) will manifest as differentiated thyroid carcinomas.[2,3] The ability to differentiate between benign and malignant thyroid conditions remains a challenge. Currently, the most cost-effective and accurate method of determination involves the performance of fine-needle aspiration (FNA).[2]

FNA is a simple and reliable procedure that is accepted as a standard method for diagnosing a thyroid nodule. However, there are limitations in characterizing cellular

The authors declare no conflict of interest.

* Corresponding author. Tel.: +1-248-231-9088; fax: +1-773-834-5295.

E-mail address: snagar29@gmail.com

Manuscript received December 12, 2012; revised manuscript June 10, 2013

0002-9610/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.amjsurg.2013.06.012

architecture, and, thus, malignancy cannot be assessed. The Bethesda System was developed to further stratify indeterminate lesions.[4] However, only 20% of patients with inconclusive pathologic findings will have follicular thyroid carcinoma (FTC) on surgical pathology.[3] The ability to identify molecular markers from analysis of FNA samples would be a useful diagnostic tool that could accurately differentiate follicular thyroid adenoma (FTA) from FTC before surgical intervention and could ultimately save the morbidity and cost associated with surgery.

Because an FNA sample has a small cell yield, a sensitive test such as quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) may be a method that could prove practical in the clinical arena because it can quickly detect gene expression with limited amounts of material. Previous studies conducted on various thyroid tissue specimens have established that qRT-PCR of different genes may differentiate benign from malignant follicular lesions.[5–10] Foukakis et al[10] evaluated 26 molecular markers in thyroid tissue using qRT-PCR and proposed 2 models implementing specific genes (including human telomerase reverse transcriptase [*hTERT*] and trefoil factor 3 [*TFF3*]) to determine benign versus malignant expression patterns. Notably, most of the studies using this method did not use an FNA sample, which limits the use of this technique during preoperative assessments.

After an extensive literature review, we identified studies that investigated specific gene models that are potential candidate markers to distinguish FTC from FTA. *hTERT* is a ribonucleoprotein polymerase that directs chromosomal telomerase and is down-regulated in normal cells. However, it has been implicated in tumor growth in both malignant thyroid tissue and FNA samples.[5–7,11,12] *TFF3* is a family of peptides with a 3-loop trefoil domain that are mainly synthesized and secreted by epithelial cells. Although the function of these proteins is not yet well understood, previous studies have linked decreased expression to thyroid follicular carcinoma.[8,9] A third biomarker, high-mobility group A2 (*HMGA2*) proteins, is a group of DNA-binding proteins that regulate gene transcriptional activity during embryogenesis. The expression of these proteins is low in normal tissue; however, a high expression has been implicated in various malignancies including thyroid neoplasia.[13,14]

By using potential biomarkers for thyroid carcinoma that have been identified in previous studies (*hTERT*, T/G ratio, and *HMGA2*), we sought to determine whether genetic expression could provide diagnostic accuracy in distinguishing FTA from FTC using prospectively collected samples. This modality may be useful in the clinical setting, and in the future, it may serve to direct more targeted future therapies for the thyroid nodule patient.

Patients and Methods

The study was approved by our institutional review board, and consent was obtained from all patients before testing. From October 2009 to June 2011, we included in our study all consecutive patients with thyroid nodules showing a follicular lesion of undetermined significance or atypical cells on undetermined significance, follicular neoplasm, and suspicion for malignancy with one of the following postoperative pathologies: follicular or Hürthle cell adenoma, follicular or Hürthle cell carcinoma, or papillary carcinoma of follicular variant (FV-PTC). Patients were recommended surgical intervention in the form of thyroid lobectomy, total thyroidectomy, or lobectomy followed by completion thyroidectomy based on current guidelines and a discussion between the patient and surgeon. Immediately after surgical resection of the specimen, an FNA with a 22-G needle was performed by the surgeon on the ex vivo thyroid tumor in the operating room. To distinguish tumor from normal tissue, gross specimens were obtained by visual inspection and determined by pathology staff. All specimens were placed in RNAlater (Qiagen, Valencia, CA) at the time of retrieval and stored at −80°C until study. Histopathologic diagnosis of all specimens was then confirmed by an independent pathologist.

Genetic markers

The following predesigned TaqMan gene-specific primers (Applied Biosystems, Foster City, CA) were used: *HMGA2* (assay ID Hs00171569m1), *TFF3* (assay ID Hs00173625m1), *hTERT* (assay ID Hs00162669m1), 3-galactoside-binding lectin (assay ID Hs00173587m1), and gene glyceraldehyde-3'-phosphate dehydrogenase (GAPDH) (assay ID Hs99999905m1). These genes were selected based on previous studies showing promise in the stratification of follicular carcinomas and adenomas.[5,6,8,10,13,15]

RNA extraction and reverse transcription

Tumor tissues were prepared for RNA extraction by Polytron (Polytron 1200, Kinematica AG, Lucerne, Switzerland) homogenization of 5 mg tissue. FNA aspirates were strained through a 35-μm cell strainer (BD Biosciences, San Jose, CA) and then centrifuged at 150*g* for 1 minute. Retained cellular material from the FNA or tumor tissue was then subjected to RNA extraction using an RNeasy Mini Kit (Qiagen, Valencia, CA). Complementary DNA (cDNA) was then synthesized by reverse transcription using 40 to 100 ng RNA with 250 ng/μL random hexamers and 200 U SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

Quantitative reverse-transcriptase polymerase chain reaction

Secondary to the limited amount of cDNA available, samples were preamplified before amplification (Applied Biosystems). In brief, this was performed by incubating cDNA with TaqMan PreAmp MasterMix (Applied