Proteomic analysis of fine-needle aspiration in differential diagnosis of thyroid nodules

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Thyroid nodules are common in the general population and vary widely in their propensity to harbor thyroid malignancies. The category of follicular lesion of undetermined significance, for instance, carries only a 15% risk of malignancy. The overarching aim of this work was the proteomic study of thyroid cancer because more effort needs to be placed on differentiating malignant thyroid nodules to avoid unnecessary thyroidectomy. We used 2-dimensional electrophoresis coupled to nano-liquid chromatography electrospray ionization tandem mass spectrometry, to examine fine-needle aspiration (FNA), which was easily attainable from the wash of the syringe used for classical FNA biopsy. Overall, we found 25 different proteins able to discriminate benign from malignant samples. The different expression of moesin; annexin A1 (ANXA1); cornulin (CRNN); lactate dehydrogenase; enolase; protein DJ-1; and superoxide dismutase was confirmed in FNA by enzyme-linked immunosorbent assay or Western blot. Receiver operating characteristic curves were calculated to investigate the discriminative power of our marker. The best performance in diagnosis was obtained by combining ANXA1, enolase, protein DJ-1, superoxide dismutase, and CRNN. In addition, the most highly ranked proteins, from the perspective of follicular lesion of undetermined significance, were ANXA1 and CRNN. The research of these candidate biomarkers has then been widened to other biological fluids, such as serum and whole saliva. In conclusion, we believe that when a decision by a thyroid nodule biopsy cannot be distinctly made, the combination of our biomarkers may be one of the criteria to be taken into account for the final decision, together with the identification of ANXA1 in serum and saliva. (Translational Research 2016;176:81–94)

Abbreviations: FLUS = follicular lesion of undetermined significance; FNA = fine-needle aspiration; FNAB = fine-needle aspiration biopsy; PTC = papillary thyroid cancer; 2DE = two-dimensional electrophoresis; NanoLC-ESI-MS/MS = nano-liquid chromatography electrospray ionization tandem mass spectrometry; WS = whole saliva; FTC = follicular thyroid carcinoma; MTC = medullary thyroid carcinoma; FT4 = free thyroxin; FT3 = free triiodothyronine; TSH = thyrotropin; TPOAb = antithyroperoxidase; TgAb = antithyroglobulin; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ANXA1 = annexin A1; MSN = moesin; CRNN = cornulin; MDH = malate dehydrogenase; LDH = lactate dehydrogenase; ENO1 = enolase; DJ1 = protein DJ-1; SOD = superoxide dismutase

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AT A GLANCE COMMENTARY

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Background

The differential diagnosis of cytologically indeterminate thyroid nodules represents a diagnostic dilemma for clinicians, this means that surgical removal of undetermined thyroid nodules is merited to rule out malignancy but is frequently unnecessary. Hence, more effort needs to be placed on differentiating benign from malignant thyroid nodules.

Translational Significance

Our results obtained in follicular lesion of undetermined significance can be added to other evidence giving the possibility of finding a method suitable to distinguish malignant from benign thyroid tumors in presurgical diagnosis, and consequently, in better understanding the mechanisms that control thyroid tumorigenesis.

INTRODUCTION

Thyroid nodules are common in the general population, and they vary widely in their propensity to harbor thyroid malignancies. Their prevalence depends largely on the method of screening and the population evaluated. Ultrasound and autopsy studies demonstrated nodules in more than 50% of women and 20% of men aged more than 50.[1-3] Among the various nodules, thyroid cancer is quite uncommon. Both ultrasound-guided fine-needle aspiration biopsy (FNAB) and cytologic interpretation of the aspirate specimen have a basic role in the diagnosis of thyroid nodules. In 2009, the Bethesda System for Reporting Thyroid Cytopathology refined cytologic definitions to improve communication and clinical management.[4] This recommended that each FNAB report begins with 1 of 6 general diagnostic categories: nondiagnostic, benign, atypia of undetermined significance/follicular lesion of undetermined significance (FLUS), follicular neoplasm or suspicious for follicular neoplasm, suspicious for malignancy, or malignant.[1,5] A meta review of 11 large studies form the United States of America showed that a median of 72% (range 62–85%) of FNAB undertaken were benign, 5% (range 1–8) were malignant, 17% (10–26) were indeterminate, and 6% (1–11) were nondiagnostic. A median of 34% (range 14–48) of patients with indeterminate cytology who underwent surgery had a malignancy.[6] In particular, the differential diagnosis of cytologically indeterminate thyroid nodules represents a diagnostic dilemma for clinicians. The indeterminate FNAB are divided in 3 categories: atypia of undetermined significance/FLUS, with malignancy in 5–10%; follicular neoplasm or suspicious for follicular neoplasm, with malignancy in 20–30%; and suspicious for malignancy, with malignancy in 50–75%.[7]

This means that surgical removal of undetermined thyroid nodules is merited to rule out malignancy, but it is also frequently unnecessary. In addition, the serious complications of hematoma, hypocalcemia, recurrent laryngeal nerve palsy, and hypothyroidism are relatively common side effects of thyroid lobectomy,[8,9] which may not be given enough weight when making a decision to perform a diagnostic lobectomy. Hence, more effort needs to be placed on differentiating benign from malignant thyroid nodules.

In this perspective, proteins are excellent targets in disease diagnosis and can be extracted from serum, tissues and cell cultures for analysis with a variety of techniques. Over the last decade, proteomics has made significant headway in addressing potential thyroid biomarkers.

In our previous studies, we applied proteomics approach to investigate the global changes in fine-needle aspiration (FNA) in papillary thyroid cancer (PTC).[10,11] With FNA, we do not refer to the classical FNAB. Indeed, the processed fluid was easily attainable from the wash of the syringe used for classical FNAB; immediately after the FNAB procedure on thyroid nodules, the syringe was rinsed with saline solution and the eluant used.

First, we investigated FNA collected immediately after total thyroidectomy,[10] and subsequently, we used immune assays to examine the applicability of results obtained on the surgical specimens on a larger number of presurgical thyroid FNA samples.[11] Our studies were focused on papillary subtypes and allowed us to highlight proteins with useful and critical insights into the molecular mechanisms of papillary thyroid tumors.

On the wave of these encouraging results, the aim of the present study was to find in FNA potential biomarkers able to differentiate benign nodules from the malignant ones, including in the analysis various tumor subtypes, not only PTC.

Therefore, we performed a comparative proteome analysis on presurgical FNA with 2-dimensional electrophoresis (2DE) coupled to nano-liquid chromatography electrospray ionization tandem mass spectrometry (NanoLC-ESI-MS/MS). The most promising biomarkers were validated in FNA, via Enzyme-linked immunosorbent assay (ELISA), to confirm the identity of proteins, and their differential expression in the classes of comparison. Moreover, the research of these candidate biomarkers was widened to other biological