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# Preoperative Diagnosis of Thyroid Carcinomas by Aspiration Biopsy-Reverse Transcription-Polymerase Chain Reaction (ABRP): A Report of Two Cases

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**Abstract.** A preoperative molecular-based diagnostic technique for thyroid papillary and anaplastic carcinomas was recently developed to detect oncofetal fibronectin (onfFN) messenger RNA (mRNA) in fine needle aspiration biopsy (FNAB) by means of reverse transcription-polymerase chain reaction (RT-PCR). We report two cases of thyroid tumors, in which cytological diagnosis was not successful but in which RT-PCR analysis provided information that helped to identify the biological features of the tumors. One case could not be diagnosed by aspiration biopsy cytology (ABC) because of poor fixation; however, RT-PCR did detect the expression of onfFN mRNA, which suggested the existence of papillary carcinoma cells. After surgery, this tumor was diagnosed as a papillary carcinoma by histological examination. The other case was diagnosed as a papillary carcinoma by ABC, but onfFN mRNA was not detected in the FNAB by RT-PCR. The neoplasm was diagnosed as a follicular tumor by histological examination. These cases suggest the benefits of combining both genetic and cytological approaches to the examination of FNAB.

*Key words*: Thyroid carcinoma, Reverse transcription-PCR, Fine needle aspiration biopsy, Oncofetal fibronectin
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**THYROID** tumors are often diagnosed by Fine Needle Aspiration Biopsy (FNAB), and cytological examinations of FNAB by a skilled pathologist who is expert in thyroid tumors provide the most reliable information for diagnosing thyroid neoplasms [1]. In some clinical situations, however, accurate cytological examinations are impossible because of the inadequacy of the slide and because diagnosis by an expert pathologist is not always available. In such cases, when a more objective method is needed to assure reliable diagnosis, reverse transcription-polymerase chain reaction (RT-PCR) analysis using RNAs extracted from FNABs may be used for this purpose. To establish a method of preoperative diagnosis of thyroid carcinomas, we introduced a new technique, called Aspiration Biopsy-RT-PCR (ABRP), for conducting simultaneous cytological and molecular-based diagnoses using leftover cells from the needle used for FNAB in a RT-PCR analysis [2]. ABRP augments the results of cytological diagnosis with data from an RNA analysis without any additional invasion of the patient.

Restricted expressions of oncofetal fibronectin (onfFN) messenger RNA (mRNA) in thyroid papillary and anaplastic carcinomas have recently been reported. onfFN mRNA can almost always be detected in tissues of these carcinomas [3–5]. Using ABRP to detect onfFN mRNA, we established an efficient method for molecular-based diagnosis of these two types of carcinomas, and the accuracy of

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this method was determined to be almost equivalent to that of the cytological examinations of a skillful cytopathologist. In a previous study, the sensitivity and specificity of this method were reported to be 96.9% and 100%, respectively, whereas those of cytological examination were 100% and 93.8%, respectively [6]. During the clinical trials of ABRP in 155 patients, we observed two cases, in which cytological diagnosis was not successful, whereas the preoperative RT-PCR analysis gave correct information on the clinical features of the tumor. In this paper, we describe these cases and discuss the clinical usefulness of ABRP.

## Subjects and Methods

### *Molecular-based diagnosis by ABRP*

Aspirates from thyroid tumors were obtained by either blind or ultrasound-guided FNAB (UG-FNAB) [7]. A syringe with a 22-gauge needle was used. RNAs were collected after informed consent was obtained. After a sample was prepared on a glass slide for cytological examinations, leftover cells inside the needle were lysed rapidly and washed out with a denaturing solution containing 4 M guanidine thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol into a 1.5 ml tube [8]. Then the tube was stored at 4 C. After the extraction of total RNA, reverse transcription was performed using the whole RNA extracted in an RT mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, 500 $\mu$M deoxynucleotide triphosphates (dNTPs), 200 U M-MLV reverse transcriptase (Gibco, Gaithersburg, MD), 2 U/ml RNase inhibitor (Takara, Shiga, Japan), and 2.5 $\mu$M oligo dT (Gibco) in a total volume of 20 $\mu$l at 37°C for 60 min. One $\mu$l of first strand cDNA was used as a template for the PCR reaction with specific primers for either onfFN or thyroglobulin. For the amplification of onfFN cDNA, a poly A-anchor primer was used instead of a 3′ specific primer to prevent false positive results. The oligonucleotides used as primers were [9, 10]:

Thyroglobulin 5′ (5′-GTTGGCAACCTCATCGT) (base 7011–7027)

Thyroglobulin 3′ (5′-AATTCTGCAGTGCCTGGT) (base 7657–7674)

onfFN 5′ (5′-AAGGCATAGGCCAAGACCATAC) (base 6127–6148)

onfFN 3′ (5′-ATGCGAATTCGTTTTTTTTTTT-TTTTTTTT)

All primers were purchased from Gibco. Each reaction mixture consisted of 1 $\mu$l of cDNA, 0.5 $\mu$M of each primer, 1 $\mu$l of 10 × Ex *Taq* Buffer, 0.8 $\mu$l of 2.5 mM dNTP mix, 0.5 U of Ex *Taq* polymerase, and nuclease-free water to a final volume of 10 $\mu$l. 10 × Ex *Taq* Buffer, dNTP mix, and Ex *Taq* polymerase were obtained from Takara (Shiga, Japan). The reaction mixture was subjected to 35 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min) and extension (72°C, 1 min). After PCR amplification, the reaction mixture was run on a 1% SeaKem GTG agarose gel (Takara). The gel was then stained with ethidium bromide (Fig. 2).

### *Case Histories*

Case 1

A 29-year-old woman with a hard nodular goiter visited our hospital. A thyroid tumor 1.2 cm in diameter was found in the isthmus by ultrasonography. FNAB in the blind manner was performed three times because the first and second FNA were not

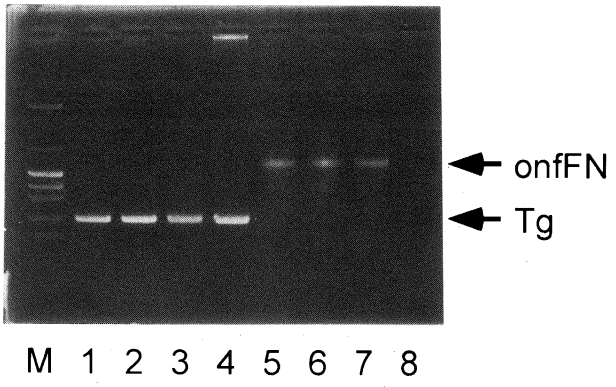


**Fig. 1.** Gel image of ABRP analysis. RNAs from FNABs was amplified by RT-PCR using specific primers for either thyroglobulin (lanes 1–4) or onfFN (lanes 5–8) cDNA, then the PCR products were run on a 1% agarose gel. The arrows indicate the expected positions of PCR products (Tg: thyroglobulin, onfFN: oncofetal fibronectin). M: PHY DNA maker (Takara), lanes 1 and 5: an aspirate from a papillary carcinoma as a positive control; lanes 2 and 6: the first FNAB from case 1; lanes 3 and 7: the second FNAB from case 1; and lanes 4 and 8: the FNAB from case 2.

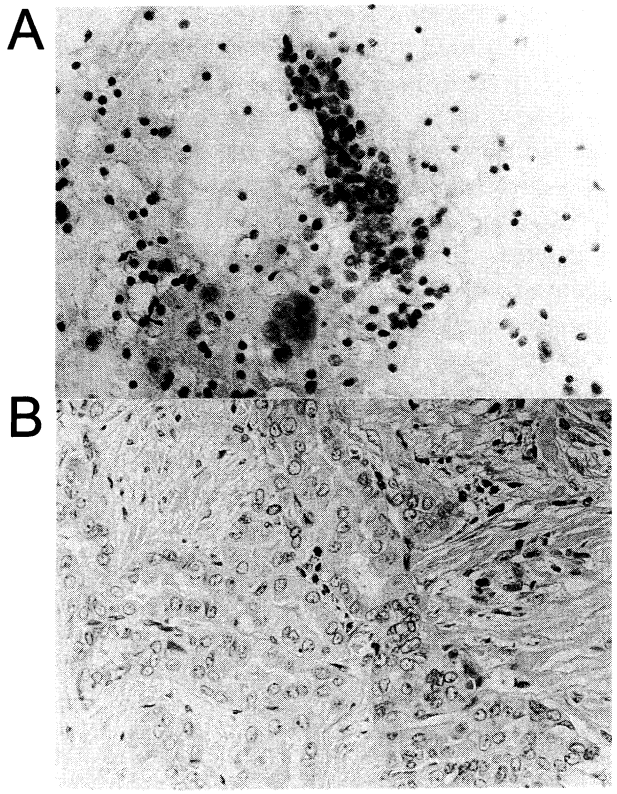


**Fig. 2.** The smear of the first FNA (Papanicolaou staining, magnification × 400) (A) and the tissue section (hematoxylin and eosin, magnification × 400) of case 1.

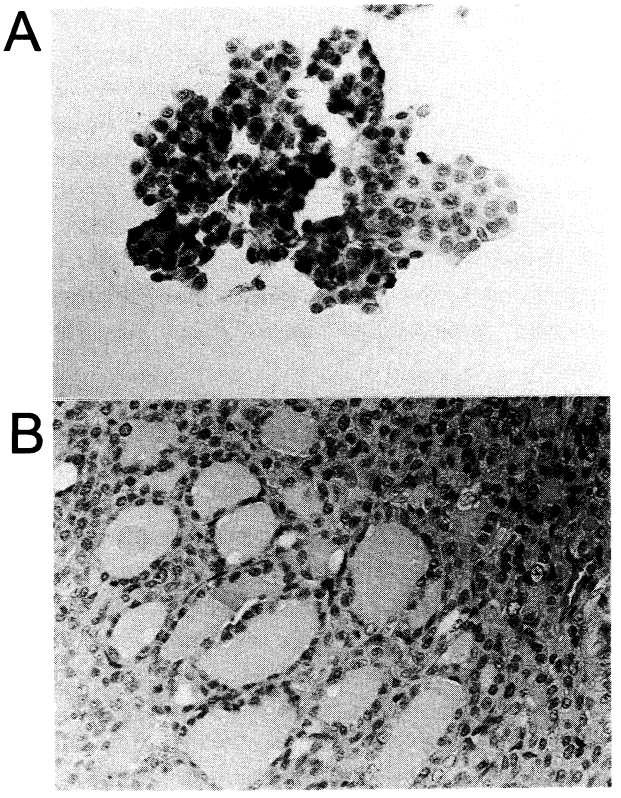


**Fig. 3.** The smear of the FNA (Papanicolaou staining, magnification × 400) (A) and the tissue section (hematoxylin and eosin, magnification × 400) of case 2.

successful due to the scant number of follicular cells and poor fixation, whereas the ABRP analysis of both FNABs showed the expression of onfFN mRNA (Figs. 1, 2). The tumor was believed to be a papillary carcinoma by the third FNAB, and subsequently, the patient underwent surgery. The tumor was then diagnosed as a papillary carcinoma. This carcinoma was rich in connective tissue, which was regarded as one of the causes of the unsuccessful aspiration.

Case 2

Ultrasonography of a 52-year-old man revealed the presence a thyroid tumor 1.0 cm in diameter in the right thyroid lobe. Ultrasound-guided FNAB was performed. The tumor was thought to be a papillary carcinoma due to the overlapping and finely granular chromatin pattern of the nuclei (Fig. 3). However, the cytopathologist was not confident about his diagnosis, since the appearance of the nuclei seemed to be a little modified because some cells were dried before fixation. RT-PCR, however, detected thyroglobulin mRNA but not onfFN mRNA in the FNAB (Fig. 1). The patient subsequently underwent surgery. According to histological examinations, this tumor was diagnosed as a follicular tumor because nuclear atypia was not evident in the tissue sections (Fig. 3). It was not determined if there were any invasions to the capsule or blood vessels, because the majority of the tumor was necrotic. Thus it was not clear whether this tumor was a carcinoma or an adenoma. Many inflammatory changes were observed in the tumor, which were likely to have been a cause of the nuclear atypia in the aspirated cells.

## Discussion

FNAB has been used as a common method for preoperative diagnosis of thyroid nodules, and its safety and reliability have been established by many studies. The sensitivity and specificity of FNAB in the previous reports, however, varied from 65 to 98%

and from 72 to 100%, respectively, due, at least in a part, to the varied skills of the cytopathologists [11]. Further, about 10 to 30% of the samples were determined to be inadequate for cytological examination because of poor fixation or insufficient number of tumor cells. In these cases, repeated FNABs are needed until adequate materials are obtained [12]. Objective diagnosis by means of a molecular-based analysis may solve these problems and provide more accurate preoperative information on thyroid tumors.

In case 1 of this study, the first and second FNABs yielded cytologically insufficient materials because of poor fixation and the scant number of tumor cells. This happened because the tumor was a hard and small mass rich in connective tissue and because the aspiration was performed in a blind manner without using ultrasonography. On the other hand, RT-PCR analysis of the same FNABs revealed the expression of onfFN mRNA, which suggested the existence of carcinoma cells. This result shows the reliability of the molecular-based diagnosis when only a small number of tumor cells are obtainable. Furthermore, as long as the tumor cells are lysed rapidly in the denaturing solution, even the analysis of dried or degraded samples is possible with RT-PCR.

Case 2 was preoperatively diagnosed as a papillary carcinoma, but after the operation the tumor was found to be of a follicular type. In this case, repeated FNAB may have been needed because a small papillary carcinoma, but not a follicular tumor, is usually indication for an operation. However, because of the mild atypia observed in the aspirated cells, it was hard to make a definite diagnosis of this tumor by cytological examination alone. To repeat the smear, rather than to operate, may be recommended when the cytological and genetic diagnoses yield discrepant results.

All procedures of ABRP, including producing both a glass slide and RNA samples, take only a few minutes, and the total cost of a RT-PCR analysis of both thyroglobulin and oncofetal mRNAs is cheaper than that of most other tests for thyroid carcinomas. Further, even though failure to detect onfFN sometimes occurs when blood and cystic fluid is aspirated simultaneously, ABRP shows high diagnostic accuracy comparable to that of a cytological examination. Therefore, RT-PCR detection of oncofetal fibronectin mRNA may be used for the first screening of papillary and anaplastic carcinomas. Multiple aspirations from a single nodule taken during one session, which has been recommended in order to prevent false positive and negative results, may not be necessary when a combined diagnosis using genetic and cytological approaches is possible [12].

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