Atypical adenoma of the thyroid diagnosed as anaplastic cancer by cytopathology

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Abstract

Atypical adenoma of the thyroid is a rare form of tumor, and its accurate diagnosis prior to surgical resection is difficult as the histological and pathological morphology are very similar to those of anaplastic thyroid carcinoma (ATC), and its anaplastic transformation remains to be elucidated.

We reported a case of a 75-year-old female with a thyroid isthmus nodule diagnosed repeatedly by FNAC as anaplastic carcinoma. Both the first and second FNAC specimen slides showed a large number of scattered or aggregated atypical cells consisting large, pleomorphic nucleoli with irregular membranes, chromatin clumps and prominent nuclei. Total thyroidectomy with central neck dissection was performed on the basis of the FNAC diagnosis. Morphology of the surgical specimen was similar to an anaplastic carcinoma, but it showed transition form from normal follicular epithelium, no invasive growth nor division of the nucleus. This lesion was diagnosed as an atypical adenoma and it contained papillary carcinoma in the right lobe.

This report evaluated the molecular features of the atypical adenoma in comparison with 9 samples of ATC, and discussed whether or not atypical adenoma was a premalignant lesion. Ki-67 expression was found to be very low

in atypical adenomas whereas all ATC samples showed high levels of Ki-67 expression. Epithelial-mesenchymal transition (EMT) marker expression suggested that atypical adenomas maintained their epithelial phenotype to a higher degree than did ATCs.

Differential diagnosis between ATC and atypical adenoma is difficult only by the cytological and histological diagnosis, Ki-67 and EMT marker expression may support the diagnosis.

Introduction

Atypical adenoma of the thyroid is a rare type of benign tumor categorized as a variant of follicular adenoma, and its morphologic features resemble those of certain malignancies. (1) (2) It is, therefore, difficult to accurately diagnose atypical adenoma using fine-needle aspiration cytology (FNAC). If we diagnosed definitely as atypical adenoma prior to surgery, surgical resection were needed because of their malignant transformation to anaplastic thyroid carcinoma (ATC). We report a rare case of atypical adenoma which was twice diagnosed by FNAC as ATC and coexisted with a papillary carcinoma. We also discuss the potential for malignant transformation by a comparison of the expression of Ki-67, epithelial-mesenchymal transition (EMT) markers and a number of growth factors in atypical adenomas and ATC samples.

Patient

A 75-years-old female had been diagnosed with hyperthyroidism at 55 years of age and subsequently treated with thiamazole for 8 years. Her thyroid function was thereafter improved and the administration of thiamazole had been stopped 12 years. She had felt abnormal sensation in the throat and visited the local

hospital 28 days prior to presentation. She was diagnosed with anaplastic thyroid carcinoma by fine-needle aspiration cytology (FNAC) and referred to our institute.

Serum levels of FT3, FT4 and TSH were within normal ranges and the serum thyroglobulin level was 17.1ng/ml. Serum anti-thyroid peroxidase antibodies (anti-TPO antibodies), thyrotropin receptor antibodies (TRAbs) and thyroglobulin antibodies were negative. Ultrasonography of the thyroid revealed a 17 x 13 x 7 mm nodule with low density in the parenchymal area to the left of the isthmus (Figure 1A) and a 6 x 6 x 5 mm nodule in the upper right lobe (Figure 1B). FNAC under ultrasound guidance was again performed to confirm the cytological diagnosis. Furthermore, PET-CT scans were obtained and no distant or lymph node metastasis was detected. The maximum standardized uptake value (*SUV*) of the thyroid nodule in the isthmus was 1.6 (Figure 1C). Although a diagnosis of a benign thyroid nodule was made on the basis of the ultrasonography and PET-scan review, total thyroidectomy with central neck dissection was performed as ATC was diagnosed on the basis of the second cytological examination and the nodule in right thyroid lobe was suspected to be a papillary carcinoma. The tumor did not invade the anterior cervical muscles,

recurrent nerve or trachea. The subsequently excised specimen was processed pathologically and studied immunohistochemically.

Cytological and Histologic Findings

The slides of the first FNAC specimen obtained in the local hospital showed a lot of scattered or aggregated atypical cells consisting of large, pleomorphic nuclei with irregular membranes, chromatin clumps and prominent nucleoli (Figure 2A).

The second FNAC specimen, which was obtained in our institute, showed a lot of pleomorphic atypical cells similar to the first cytology slides, multinuclear cells and spindle cells, and a diagnosis of ATC was made on the basis of the second FNAC. The nodule in the isthmus showed a focal and palisading arrangement of tumor cells containing both prominent and pleomorphic atypical nuclei. In the peripheral area, tumor cells with smaller and rounder shape nuclear multiplied with the appearance of follicular formation. It looks like an anaplastic carcinoma but the number of spindle and pleomorphic cell in this tumor were thought to be smaller than that in typical anaplastic carcinoma. Follicular carcinoma was distinguished by the absence of thickened membrane. Follicular variant of papillary carcinoma could be one of the candidates of diagnosis by the partial

presence of cleaved cells, but it was not definitively diagnosed by the absence of intranuclear inclusion body. Atypical adenoma was the final histopathological diagnosis because: 1) it showed transition form from normal follicular epithelium, 2) invasive growth, division of the nuclei and clumped euchromatin were not observed, 3) tumor cells with deformed nuclear can be appeared in atypical adenoma. (Figure 2BC). Another nodule in the right lobe presented as a typical papillary carcinoma with a typical papillary growth pattern with finger-like projections lined with neoplastic cells. Metastatic papillary carcinoma cells were detected in 2 lymph nodes in the pre- and paratracheal area, but no anaplastic or poorly differentiated carcinoma lymph node metastasis was observed.

Molecular analysis

To investigate the molecular biological features of atypical adenoma, we undertook immunohistochemical staining with several molecular markers associated with tumor aggressiveness and compared the results obtained for atypical adenoma and 9 anaplastic cancer samples. Immunohistochemical staining data are summarized in Table 2.

Ki-67 is a nuclear protein associated with cellar proliferation through ribosomal

RNA transcription and is using as a marker for cellular proliferation. (3) Less than 1% of cells were stained in the atypical adenoma (Figure 3A), whereas 30-90% of cells in the ATC samples were stained with Ki-67 (Figure 3B).

Beta-catenin and E-cadherin are associated with cell-cell adhesion and their expression is usually used as an epithelial marker. (4) Both epithelial markers were highly expressed in the atypical adenoma (Figure 3C); however, E-cadherin expression was lost in eight of 9 ATC samples (Figure 3D) and 5 of 9 ATC samples showed low beta-catenin expression. Vimentin is used as a mesenchymal marker. Vimentin expression was low in the atypical adenoma, but 5 of 9 ATC samples showed high levels of vimentin expression.

Recently, targeted molecular therapy was developed for use against thyroid cancer to some effect. (5) (6) The EGFR and VEGF signaling pathways are most popular targets for this therapy; therefore, EGFR and VEGF expression were examined. EGFR expression was high in the atypical adenoma and in 89% of ATCs, whereas VEGF expression was moderate in the atypical adenoma and varied among the ATC samples.

Materials and Methods

Aspiration was performed using a 23-gauge needle under ultrasound guidance, and the alcohol-fixed smears were papanicolaou stained. The thyroidectomy specimen was embedded in paraffin and stained with hematoxylin and eosin, prior to cutting into 4-mm sections. Following microwave pretreatment in a citrate buffer (pH 6.0) for 5 min (x 3) at 750 W, these sections were treated with 10% normal rabbit serum for 30 min to prevent non-specific binding of the antibody. The slides were then incubated with specific monoclonal antibodies to Ki-67, Beta-catenin, E-cadherin, vimentin, EGFR and VEGF in a humid chamber at 37.8C overnight. The sections were then incubated with a biotin-labeled rabbit anti-mouse secondary antibody (Histo-fine SAB-PO (M) kit; Nichirei, Tokyo, Japan) for 30 min at 37.8C, followed by reaction with a streptavidin-biotin horseradish peroxidase complex. Evaluation of the immunohistochemical staining of all molecular markers as performed as reported previously. (7) Vimentin, which was not included in the above, was evaluated for cytoplasmic staining semi-quantitatively. The scoring systems are summarized in Table 1. A score 0 or 1 was categorized as low expression, with a score 2 or 3 categorized as high expression. All samples were evaluated and scored by 2 investigators

(H.H, K.H) under the supervision of the Department of Pathology of Hokkaido University hospital.

Discussion

Atypical adenoma is rare and shows morphologic features very similar to those of ATC, both in cytological and histologic terms. In this case reported herein, our clinical cytologists diagnosed ATC without suggesting the possibility of atypical adenoma. Several reports have argued that there is no effective method of distinguishing between these tumors by cytology. (8, 9)Immunohistochemical analysis using Ki-67 and epithelial markers appears to provide a good option for differential diagnosis. However, the question arises as to whether accurate diagnosis prior to surgery would allow doctors to choose a wait-and-see policy. If atypical adenoma is a premalignant lesion associated with ATC, surgical resection would be needed prior to malignant transition as the prognosis for patients with ATC is generally very poor due to its rapid growth and high frequency of distant metastasis. (10) (11) Detection of the biological mechanism underlying carcinogenesis in ATC may provide more specific treatment targets. Atypical adenoma seems to be one of the best models with which to investigate the mechanism of carcinogenesis in ATC. Several reports have discussed the notion of whether ATC is derived from atypical adenoma, PTC, follicular nodules or follicular carcinoma. (7, 12) Mutation studies have shown that BRAF

mutations were detected in 29 to 69 % of PTCs and in 10 to 35 % of ATCs. On the other hand, p53 mutations were found in 0 to 5 % of PTCs and 67 to 88% of ATCs. (13, 14) (15) (16) These genetic findings suggested that carcinogenesis mechanism were different between ATC and PTC. Tzen et al. demonstrated p53 mutations in atypical adenoma and suggested that ATC was derived not from PTC, but follicular carcinoma or atypical adenoma. (9)

EMT enhances the aggressiveness of epithelial cancer cells and induces metastasis, local invasion and drug resistance. (17) Our study suggests that ATC loses its epithelial phenotype, whereas atypical adenoma maintains its epithelial phenotype. Several reports have shown that higher expression levels of ZEB1 and Snail1, which activate EMT, in ATCs than in other thyroid tumors, and EMT was associated with the aggressiveness of thyroid carcinoma. (18) (19) (20) Surgical specimens of ATCs often include non-contiguous papillary carcinoma, which is a one of the reasons that we speculate ATC is derived from PTC. Our results also suggest that ATC might have been derived from the atypical adenoma with EMT, rather than from the PTC.

Figure Legends

Table 1. Scoring systems for the markers.

Table 2. Summary of marker staining in the ATC samples and the atypical adenoma

Figure 1A. Ultrasound images of the atypical adenoma in the left isthmus along the horizontal and sagittal plane. (17 x 13 x 7 mm)

Figure 1B. Ultrasound images of the papillary carcinoma in the right lobe along horizontal and sagittal plane (6 x 6 x 5 mm)

Figure 1C. CT and PET-CT images of the atypical adenoma. The *SUV* max of the thyroid nodule in the isthmus was 1.6.

Figure 2A. Atypical cells from the FNAC specimen slides. A lot of scattered or aggregated atypical cells consisting of large, pleomorphic nuclei with irregular membranes, chromatin clumps and prominent nucleoli.

Figure 2BC. Histopathological features of the atypical adenoma. Focal and palisading arrangement of the tumor cell containing prominent and pleomorphic atypical nuclei are shown. No invasion of multiplying tumor cells can be seen in

this nodule, and imaging does not show any division of the nuclei or shortage of clumped euchromatin.

Figure 3.

Ki-67 and E-cadherin expression in the atypical adenoma and anaplastic thyroid carcinoma samples.

In the atypical adenoma, less than 1 % of cells were stained with Ki-67 (A), whereas Ki-67 was highly expressed in the ATC samples (B).

E-cadherin strongly expressed in atypical adenoma(C) and weakly expressed in ATC (D).

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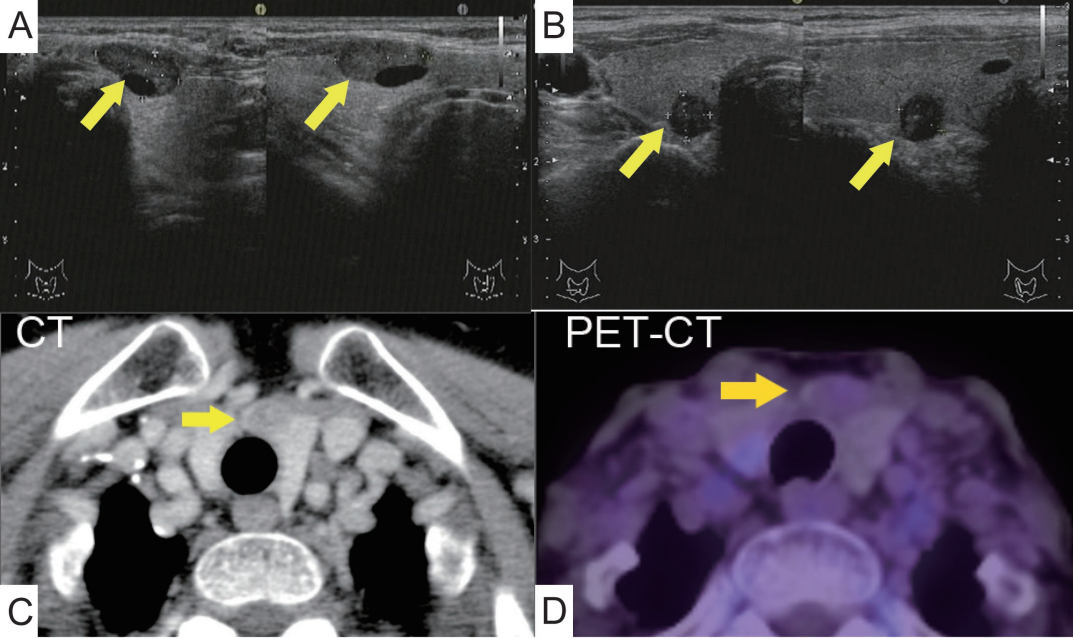
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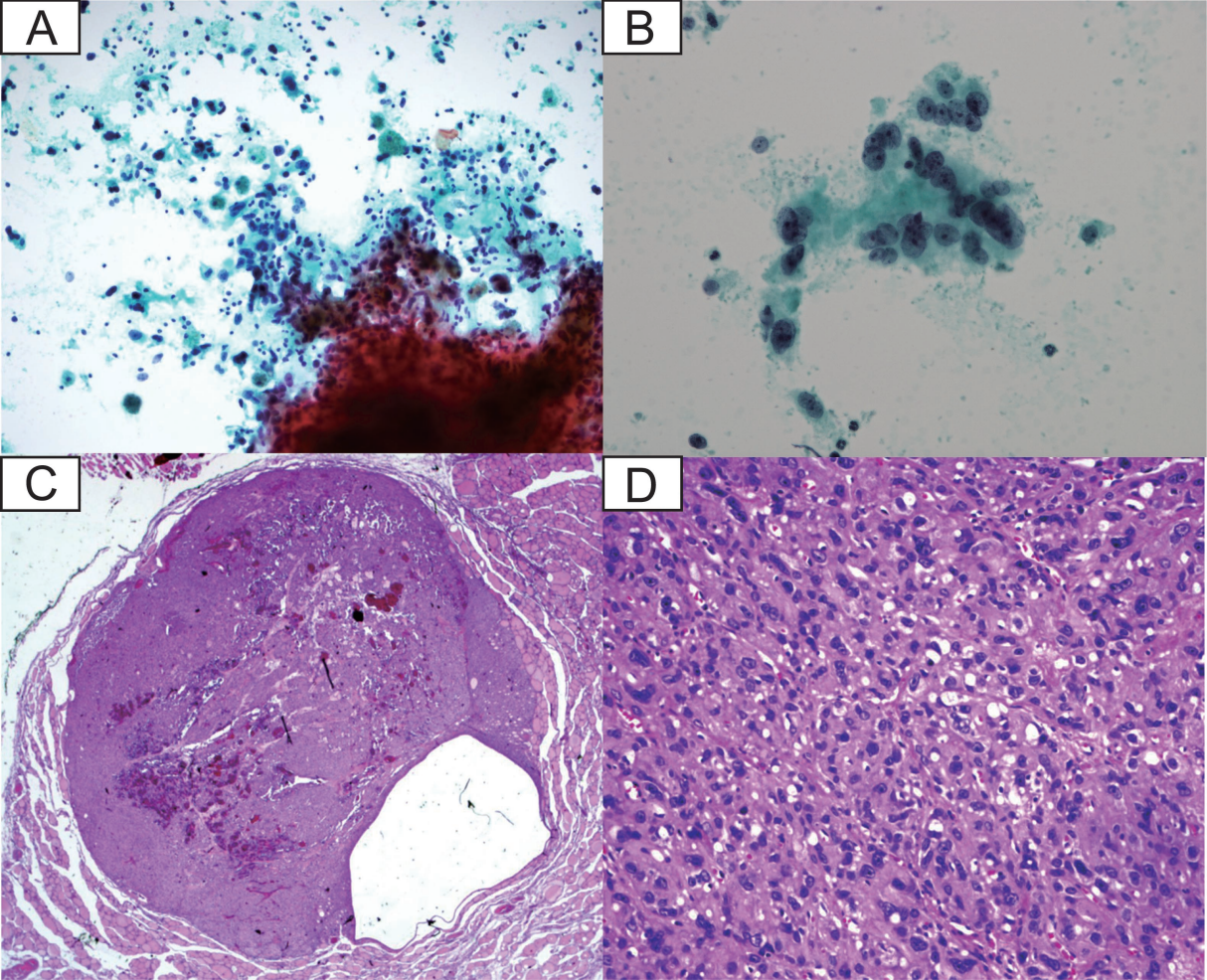
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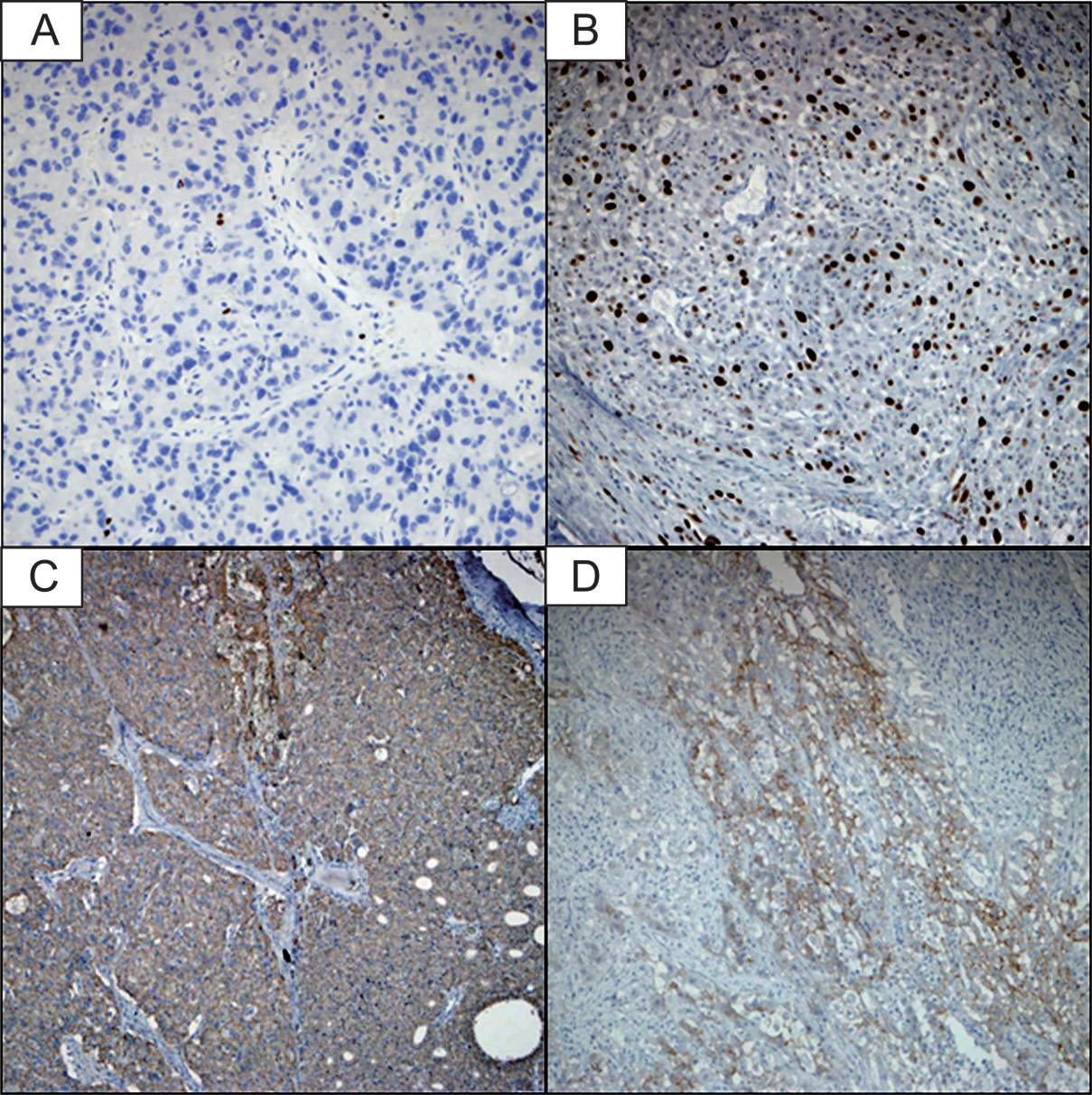
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Table 1. The Scoring Sytems for Markers Evaluted

Marker Name	Locallization	Scoring system
Ki-67	nuclear	3+=>50% Cells positive 2+=26%-50% Cells positive 1+=5%-25% Cells positive 0=<5% Cells positive
Beta-catenin	cytoplasmic ± nuclear	3+=>75% Of ells positive 2+=26%-75% Of cells positive 1+=5%-25% Of cells positive 0=<5% Of cells positive
E-cadherin	membranous	2+=Strong 1+=Weak 0=Negative
Vimentin	cytoplasmic	2+=Strong 1+=Weak 0=Negative
EGFR	cytoplasmic ± menmranous	3+=>75% Of ells positive 2+=26%-75% Of cells positive 1+=5%-25% Of cells positive 0=<5% Of cells positive
VEGF	cytoplasmic ± membranous	2+=Strong 1+=Weak 0=Negative

Table 2

	Anaplastic carcinoma (n=9)	Atypical adenoma
Ki-67	Low: 0 High: 9	Low(0)
Beta-catenin	Low: 6 High: 3	High(2+)
E-cadherin	Low: 8 High: 1	High(2+)
vimentin	Low: 4 High: 5	Low(1+)
EGFR	Low: 1 High: 8	High(2+)
VEGF	Low: 5 High: 4	Low(1+)