

## Original Article

# Analyze two cases of encapsulated papillary oncocytic neoplasms (EPONs) of the thyroid

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**Abstract:** Aim: Analyzing the pathological features of encapsulated papillary oncocytic neoplasms (EPONs) of the thyroid based on 2 cases in our department. Methods: Medical history and morphological characteristics of the two cases were reviewed. Immunohistochemical staining was applied to detect the expression of CK19, CD56, CD31, galectin-3 and so on with paraffin-embedded tissues. Sanger sequencing was performed to detect the genotype of codons V600 in BRAF and codons GG12/13 in KRAS. Results: The first patient has been diagnosed as Hurthle cell adenoma 8 years ago; the second patient discovered that the mass grew up gradually for 3 months. Oncocytic cells of EPONs mainly arranged and formed to papillary architecture but did not exhibit the nuclear feature of papillary thyroid carcinoma under microscope. Invasion of capsule and vascular vessels could be observed in case 1. The two cases of EPONs displayed CK19(-), CD56(+), galectin-3(+), Wide Genotype of BRAF and KRAS. In the follow-up, the two patients remain alive within 5 and 3 years, respectively. Conclusions: EPONs might occurring through the malignant transformation from Hurthle cell adenoma; however, this process was different from classical Hurthle cell adenocarcinoma. They were malignant tumor which didn't diagnosed on infiltrating of vessels or capsule.

**Keywords:** EPONs, oxyphilic thyrocytes, papillary, malignant transformation

## Introduction

EPONs (Encapsulated Papillary Oncocytic Neoplasms) of the Thyroid were rare tumors which defined as encapsulated thyroid tumors composed of oncocytic thyrocytes with at least focal papillary architecture. Cases demonstrating readily apparent nuclear features of papillary thyroid carcinomas (PTCs) were excluded. EPONs were very rare and their relationship with other oncocytic neoplasms of the thyroid remained unclear. In some instances, EPONs were often misdiagnosed as other neoplasms with a similar pattern. In this study, the clinicopathologic and molecular features of two cases of EPONs were analyzed.

## Materials and methods

Two cases of EPONs in our department were considered. All of the tissues were fixed in 10% neutral formalin, desiccated, and embedded in paraffin. A two-step immunohistochemical staining technique (EnVision) was employed to

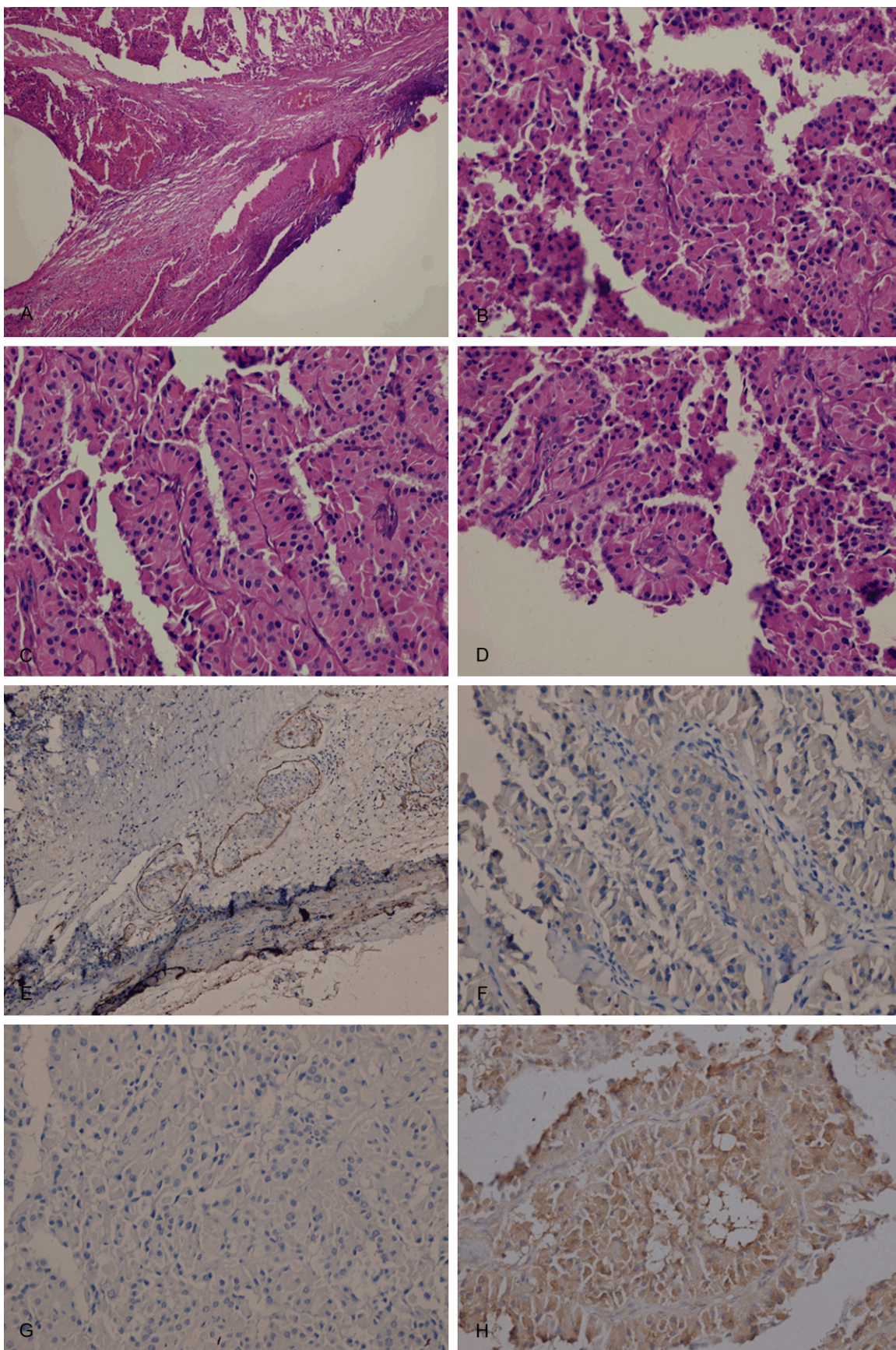
detect TG, CK19, galectin-3, CD56, calcitonin, CD31, CD34, and Ki-67. Sanger sequencing was applied to detect the gene phenotype of codons V600 in BRAF and codons GG12/13 in KRAS for the two samples.

## Results

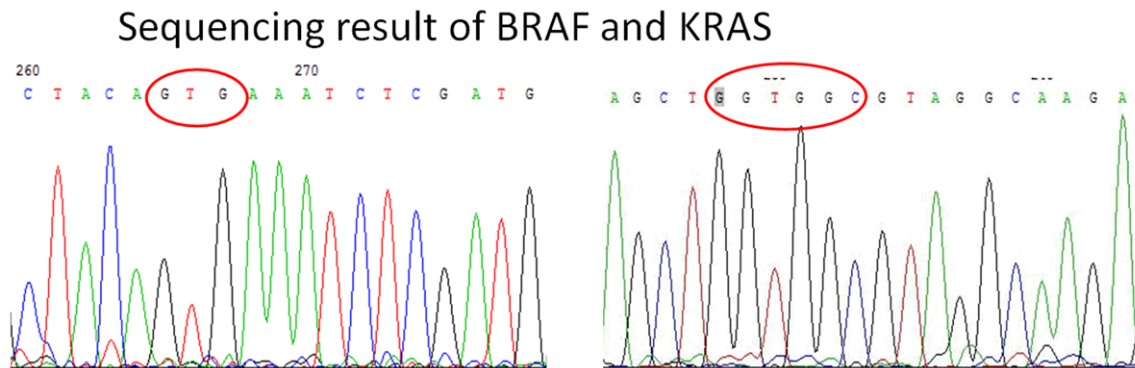
### *Introduction of illness and clinical characteristics*

**Case 1:** In 2010, a 58-year-old female patient manifested a mass in the isthmus portion of the thyroid but did not complain of hoarseness, bucking, and dyspnea. A pathologist found a pinkish-gray nodule with a dimension of 4.5×4×4 cm in the isthmus portion of the thyroid with an integrated capsule. In a follow-up, the patient remains alive and does not suffer from recurrence after the whole thyroid was resected. Past history: In 2002, the patient was diagnosed with Hurthle cell adenoma. The sections in 2002 were reviewed which displayed a typical image of Hurthle cell adenoma without pap-

## EPONs of the Thyroid



**Figure 1.** A-D: HE staining. A: Invasion of vascular in capsule for case 1 (10×); B: Papillary structure for case 1 (20×); C: Papillary structure for case 2 (20×); D: The reverse phenomenon for case 2 (20×). E-H: Immunostainings for EPONs. E: CD31 for vascular in case 1; F: CD56(+); G: CK19(-); H: Galectin-3(+).



**Figure 2.** Left: codons V600 of BRAF (Wild Genotype); Right: codons GG12/13 of KRAS (Wild Genotype).

illary structure and did not invade the vascular vessels or the capsule.

**Case 2:** A 47-year-old female patient was detected with a chicken-egg-sized neck mass 3 month ago before see a doctor in 2012. The mass grew to a size of a duck egg within a month. The patient did not suffer from heart palpitations, fever, and pain. Conservative treatment was not applicable. Color Doppler ultrasound: A regularly sized mass was detected in the right lobe of the thyroid. The mass exhibited a slightly lower echo but a non-uniform echo. A 6×5.5×4.2 cm nodule of the thyroid tissue was examined, and the pinkish-gray cross section was found. The thyroid was resected completely.

### Microscopic characteristics

The tumor cells were columnar with a granular eosinophilic cytoplasm. Most of the tumor cells were arranged in the papillary form, and the subsection was arranged in a trabecular pattern, with a solid and alveolar structure. The cells were arranged in single layer; the nucleus was partly found in the center of the cell and partly located in the top of the adenoid (the reverse phenomenon). The nuclei were round or oval with prominent nucleoli. The typical nuclear features of PTCs, such as large and crowded and translucent nuclei with nuclear groove and intranuclear pseudoinclusion, were not observed in the two cases. Small vessel invasion was found in the local area of the fiber within the capsule in Case 1 (**Figure 1A-D**).

### Molecular characteristics

Immunohistochemistry staining results displayed TG(+), CK19(-), galectin-3(+), CD56(+), calcitonin(-), and Ki-67<1% in the two cases. CD34 and CD31 were considered as markers of the invaded vascular vessels in Case 1 (**Figure 1E-H**). DNA sequencing results revealed the wild genotype of codons V600 in BRAF and codons GG12/13 in KRAS for the two samples (**Figure 2**).

### Discussion

Hurthle cell tumors were the unique type of thyroid follicular tumors that wholly or mainly (more than 75%) consist of acidophils. A polygonal cell is composed of large and rich eosinophilic granules in the cytoplasm. True papillary structure without nuclear features of PTCs were relatively rare in Hurthle cell neoplasm. Woodford RL [1] proposed that tumors with a papillary structure and acidophilia should be classified as Hurthle cell adenoma or adenocarcinoma on the basis of capsular and vascular vessel invasion. Berho M [2] found that some eosinophilic tumors with a real papillary structure contained a fiber axis but did not consist of the nuclear feature of PTC, and this condition was described as Hurthle cell tumor papillary variant.

Encapsulated papillary oncocytic neoplasms (EPONs) were firstly been named in the 1992 Atlas of Tumor Pathology (AFIP) fascicle on thyroid tumors which with papillary structure and acidophilia [3]. EPONs were rare tumors in the thyroid gland and usually classified as Hurthle

cell adenoma/adenocarcinoma or papillary carcinoma inaccurately.

We analyzed two cases of EPONs that rich in eosinophilic granules and arranged into fibro-vascular papillae. The first patient suffered from typical Hurthle cell adenoma 8 years ago and the recurrent tumor displayed papillary structure and invaded the vascular vessel in the capsule. This result indicated that EPONs were related to Hurthle cell adenoma in the origin. The second patient displayed a tumor that increased in size significantly within a month. The recent increasing in tumor size was evidently manifestation of malignant transformation. We proposed that EPONs were the special results from Hurthle cell adenomas.

Hurthle cell adenocarcinoma referred to Hurthle cell adenoma with vascular vessel or capsular invasion. Carcangiu ML [4] proposed that EPONs should be evaluated on the basis of the histological criteria of follicular tumors, including vascular or capsular invasion and distant metastasis. In case 2, all the mass was selected for dehydration and embedding but no invasion were discovered under microscope. We proposed on biological behavior that EPONs were different from Hurthle cell adenocarcinoma regardless of vascular or capsular invasion.

Galectin-3 has been implicated in the regulation of cellular growth, differentiation and malignant transformation in thyroid gland. Diffuse and strong immunohistochemistry staining for galectin-3 might potentially serve as a marker in difficult differential diagnosis cases involving Hurthle cell adenomas and Hurthle cell carcinomas [5]. Galectin-3 immunostaining in Hurthle cell carcinomas was significantly higher than in Hurthle cell adenomas [6]. The two cases all displayed Galectin-3 positive with immunostaining which indicated that EPONs were malignant neoplasms independent of tumor invasion.

KRAS mutations and heteroploid were detected more often in malignant follicular neoplasm than benign [7]. All the two cases showed codons GG12/13 of KRAS wild genotype with Sanger sequencing. We speculated that the 2 cases could not represent the whole phenomena of KRAS. Moreover, the tissues were saved too long and DNA fragment might have been fractured in same degree. In addition,

mutated tumors less than 20% and exon 3/4 mutation could not been detected in our study with Sanger sequencing.

EPONs were different from PTCs because of two features: morphological characteristics and molecular biomarkers. Despite of the actual papillary structure, EPONs didn't possess the characteristics of the nucleus similar to that of PTCs. PTCs usually displayed CK19(+), HBME-1(+), and CD56(-). Polymerase chain reaction test results revealed that 69% of PTCs displaying gene mutation in BRAF [8]. Bellevicine C [9] subjected EPONs to immunostaining and found CK19(-), HBME-1(-), and CD56(+). Woodford RL [1] detected nine cases of EPONs but did not find BRAF gene mutation. These results were not consistent with that observed in PTCs. Our immunostaining results also showed that the two cases were CK19(-)/CD56(+). DNA sequencing results demonstrated that codons V600 in BRAF were wild genotype within the two cases.

Differential diagnosis mainly includes the following: (1) Hurthle cell adenomas: EPONs showed cytological characteristics similar to Hurthle cell adenoma, but EPONs were arranged at least focally to typical papilla with true fiber axis. Hurthle cell adenomas does not invade the envelope and vascular vessels. (2) Hurthle cell adenocarcinomas: Large tumor cells contained a large nucleus and exhibited heteromorphism. Large acreage of the papilla was missing. Vascular vessel/capsular invasion or distant metastases were the diagnostic morphology features for Hurthle cell adenocarcinomas but didn't appropriate for EPONs. (3) Subtype of PTCs with an acidophil type: This conditions exhibited the typical characteristic of the nucleus. PTCs were characterized by glass-like nucleus, with thick nuclear membrane and intranuclear pseudoinclusions. Molecular biomarker revealed CK19(+), HBME-1(+), CD56(-) and mutation of BRAF gene. (4) Other tumors: Other tumor types, such as PTC with a high cell type, medullary carcinoma with an acidophilic cell type, and metastatic tumors.

### Summary

We considered that EPONs were included in Hurthle cell neoplasms. They might the unique results of malignant transformation from Hur-

thle cell adenomas and different from classical Hurthle cell adenocarcinomas. Diagnosis of EPONs mainly based on the pathological morphology independent of tumor invasion. Immunostaining results displayed CK19(-), CD56(+), and galectin-3(+). The BRAF gene always showed wild genotype in EPONs. EPONs should be treated with the same strategies as those used to cure invasive tumors and closely following up was necessary [10].

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## Disclosure of conflict of interest

None.

## Authors' contribution

Ding Li: put in order of data and write the article, sequencing for BRAF and KRAS. Zou Xianjin: pathological diagnosis for EPONs and guide the work. Yang Wan: immunohistochemical staining. Xie Junhua: HE staining.

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