Malaysian J Pathol 2019; 41(3) : 339 – 343

CASE REPORT

Cytohistology of morule in cribriform-morular variant of papillary thyroid carcinoma

Kouhei SAKURAI[1], Takanori ONOUCHI[2], Seiji YAMADA[1], Youichirou BABA[3], Tetsuya MURATA[3], Tetsuya TSUKAMOTO[1], Makoto KURODA[1], Makoto URANO[1]

[1]Department of Diagnostic Pathology, Fujita Health University School of Medicine, Toyoake, Aichi, Japan, 470-1192, [2]Center for Research Promotion and Support, Fujita Health University, Toyoake, Aichi, Japan, 470-1192, [3]Department of Pathology and Laboratory Medicine, JA Suzuka General Hospital, Suzuka, Mie, Japan, 513-8630

Abstract

Introduction: Cribriform-morular variant (CMV) is a rare variant of papillary thyroid carcinoma. It frequently occurs in association with familial adenomatous polyposis (FAP), although some cases are sporadic. Herein, we report a case of CMV and analyse morule cytohistology. *Case Report:* The patient was a 47-year-old woman with no familial history of FAP. A 3.0-cm unifocal mass was identified in the left thyroidal lobe. Fine-needle aspiration cytology revealed papillary clusters of atypical cells with nuclear grooves, which was suspected to be conventional papillary thyroid carcinoma. Histologically, the tumour comprised a papillary and cribriform growth of atypical cells with cytoplasmic accumulation and nuclear translocation of β-catenin. In addition, frequent morule formation was identified. *Discussion:* In this case, we performed morule analysis through correlative light and electron microscopy (CLEM), and revealed its ultrastructure. Although CMV is a rare form of thyroid carcinoma, it should be considered along with its distinct clinicopathological characteristics.

Keywords: Papillary thyroid carcinoma, cribriform-morular variant, morule

INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common histological type of thyroid carcinoma.[1] Histologically, PTC is diagnosed by nuclear findings, including the presence of a nuclear groove and intranuclear cytoplasmic inclusion. Certain histological types of PTC exhibit diverse histological structures that are not observed in conventional PTC and are classified into variant types of PTC.[2] Among these, cribriform-morular variant (CMV) is rare, and its prevalence has been reported to be 0.16% among all types of PTC.[3] It occurs as multifocal tumours, predominantly in young women with a strong association with familial adenomatous polyposis (FAP).[2] It is suspected that a germline mutation of *adenomatous polyposis coli* (*APC*) is associated with tumourigenesis. Conversely, other cases sporadically occur regardless of FAP. In sporadic cases, the somatic mutations of *CTNNB1* coding β-catenin and *APC* are involved in tumour development. In FAP-related and -unrelated cases, the nuclear translocation of β-catenin is a characteristic of CMV as a result of *APC* and/or *CTNNB1* mutations, which suggests the activation of the Wnt/β-catenin pathway. Histologically, CMV exhibits papillary, cribriform, and follicular growth patterns.[2] In addition, morules, which comprise squamoid cells, are frequently observed and are regarded as a specific histological finding of CMV. Herein, we report a case of CMV and analyse the cytohistology.

CASE REPORT

A 47-year-old woman with no personal or family history of FAP was presented with a unifocal mass measuring approximately 3.0 cm in the left thyroidal lobe. Fine-needle aspiration cytology (FNAC) was performed, however, no atypical cells suggestive of malignancy were detected. After 10 months, a second FNAC was performed,

Address for correspondence: Makoto URANO, M.D. Ph.D., Department of Diagnostic Pathology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi, Japan 470-1192. Tel: +81-562-932-319. Fax: +81-562-95-3761. Email: uranom@fujita-hu.ac.jp

which revealed papillary cellular clusters and monolayer sheets of the epithelia comprising atypical cells with a cuboidal cytoplasm and few nuclear grooves (Fig. 1A and B); therefore, conventional PTC was suspected. Although the levels of serum thyroglobulin (39.0 ng/mL, reference range: 0–33.7 ng/mL) were marginally increased, other laboratory data were normal.

The patient was admitted to our hospital, and total thyroidectomy was performed. Macroscopically, the tumour was capsulated in the left lobe (Fig. 2A), with no tumour identified in the right lobe. Hematoxylin and eosin (HE)-stained sections from formalin-fixed paraffin-embedded (FFPE) tissues revealed that the tumour was composed of papillary (Fig. 2B) and cribriform (Fig. 2C) growths of tumour cells with a cuboidal cytoplasm. A nuclear groove was observed in the limited tumour cells (Fig. 2D). Of note, several morules were identified, which were composed of squamoid cells with nuclear clearing (Fig. 2E). Immunohistochemical analysis was performed using the amino acid polymer method, which is a biotin-free system. Positive immunoreactivity for CK7, CK19, TTF-1, ER, PgR, and CD10 and a negative immunoreactivity for CK20, thyroglobulin, and Pax-8 were observed (data not shown). p53 staining revealed a wild-type pattern, and the Ki-67 labeling index was 5.35% (data not shown). Of note, the cytoplasmic accumulation and nuclear translocation of β-catenin, which is a hallmark of CMV, was observed in the cuboidal tumour cells and certain morular cells (Fig. 2F). The case was pathologically diagnosed as CMV based on the findings mentioned above.

Retrospectively, the second FNAC specimen was re-evaluated, and a cellular cluster suggestive of a morule was identified (Fig. 3A). Visualisation of the microstructure of the morule in the FFPE sample was attempted. We have previously reported the use of correlative light and electron microscopy (CLEM), which is a system for the observation of histological microstructure in the same field of optical and electron microscopes[4]. A paraffinised section was stained with HE, freeze-dried, and observed without using a coverslip under a normal optical microscope. As shown in Fig. 3B, a morule comprising several cells with nuclear clearing was observed. The section was then sputter-coated with gold-palladium, and the same area (as the normal optical microscope observation) was examined under a scanning electron microscope (SEM) (Figs. 3C and D). Each cell exhibited a protruded cell membrane and fusion with the neighbouring cells, in a web-like manner. In addition, the nuclear region was filled with an amorphous substance, which was recognised as nuclear clearing under the optical microscope (Fig. 3B).

DISCUSSION

Of the CMV cases, >50% are complicated with FAP, predominantly occurring in young women. In the present case, following a diagnosis of CMV, we pathologist advised the clinicians to eliminate the presence of gastrointestinal polyposis although neither the patient nor the family had a history of FAP. The patient was transferred to another hospital. Thus, we were unable to follow up on the result of the gastrointestinal finding. And the gene mutations of *APC* and *CTNNB1* were not analysed in this case. However, this case may be unrelated to FAP because the age

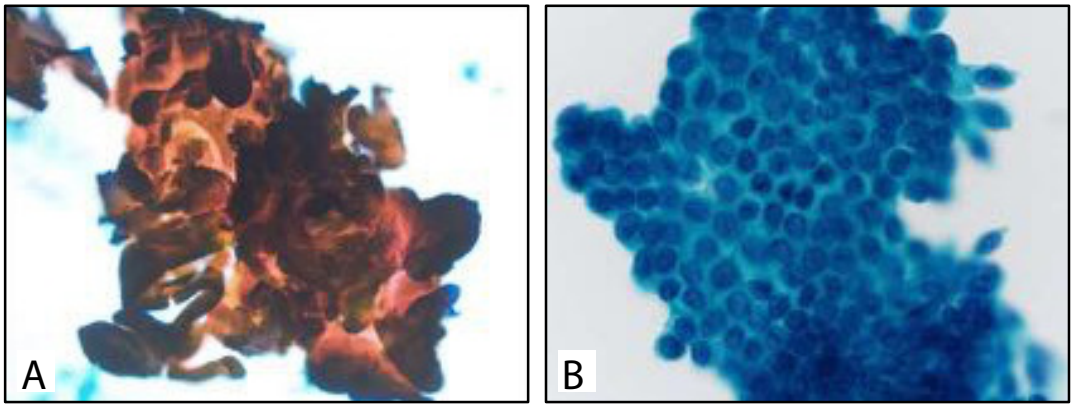

FIG. 1: Cytological findings of fine-needle aspiration cytology
A. Papillary cellular clusters (magnification, x200). B. Monolayer cellular sheets comprising several cells with few nuclear grooves (magnification x1000).

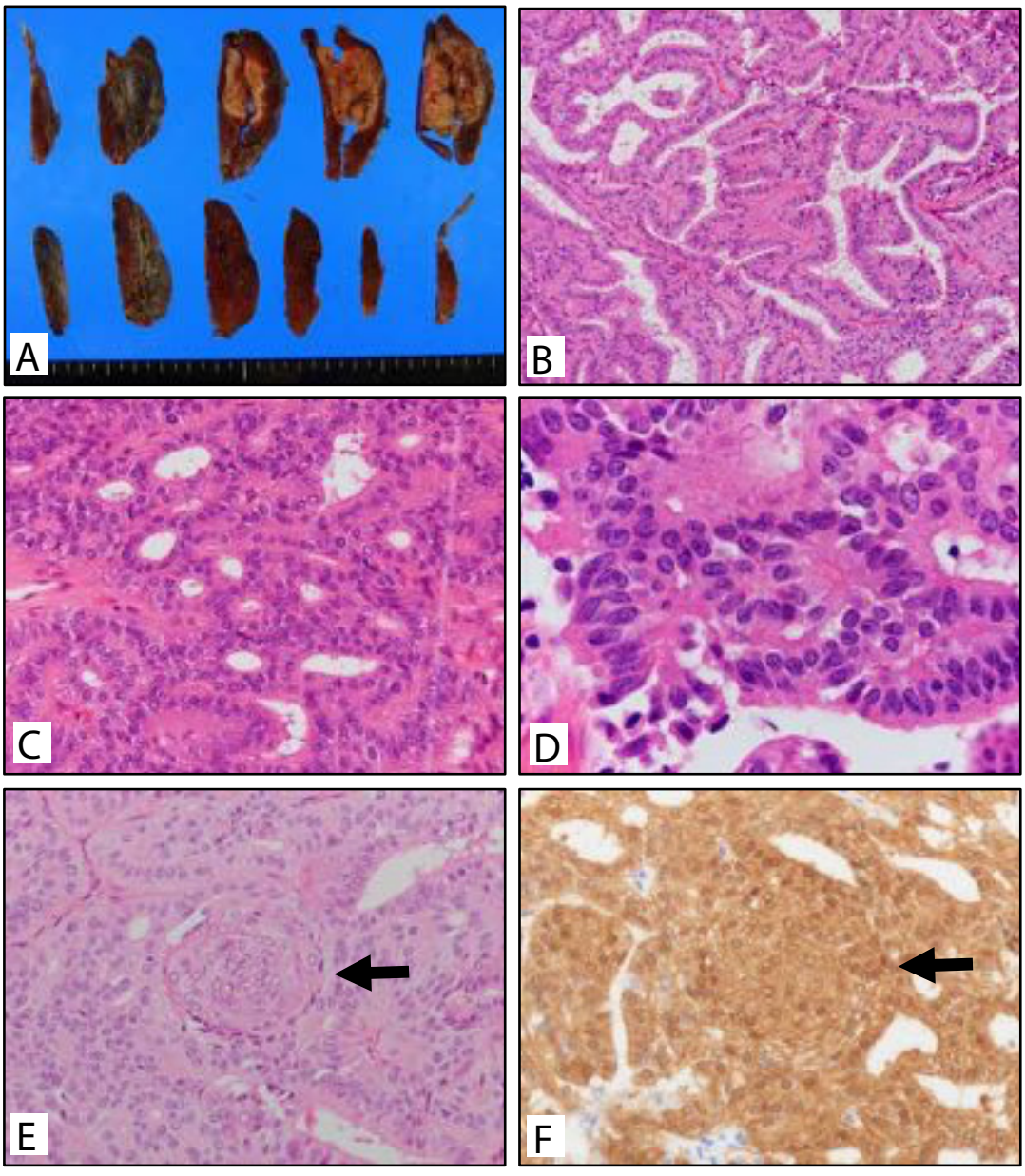

FIG. 2: Histological findings
A. Macroscopic view of this case. B. Image of HE staining of papillary growth (magnification, x200). C. Image of HE staining of cribriform growth (magnification, x200). D. Image of HE staining of atypical cells with a few nuclear grooves (magnification, x1000). E. Image of HE staining (magnification, x200) of morule. Black arrow indicates the morular growth pattern comprising cells with nuclear clearing. F. Immunohistochemistry of b-catenin (magnification, x200). Black arrow indicates a morule. HE, hematoxylin-eosin.

of the patient was relatively high (47 years), and the tumour was sporadic and unilateral, all of which are typical clinicopathological findings of an FAP-unrelated case.[2]

For comparison, immunohistochemistry of β-catenin was performed against other patients with conventional PTC, and a tall cell variant of PTC, including a case previously reported by our group;[5] however, no translocation was observed in any of the analysed cases (data not shown). In addition to the expression of β-catenin, morular formation is regarded as a hallmark of CMV. The reason why morules are formed remains unclear; however, morular formation

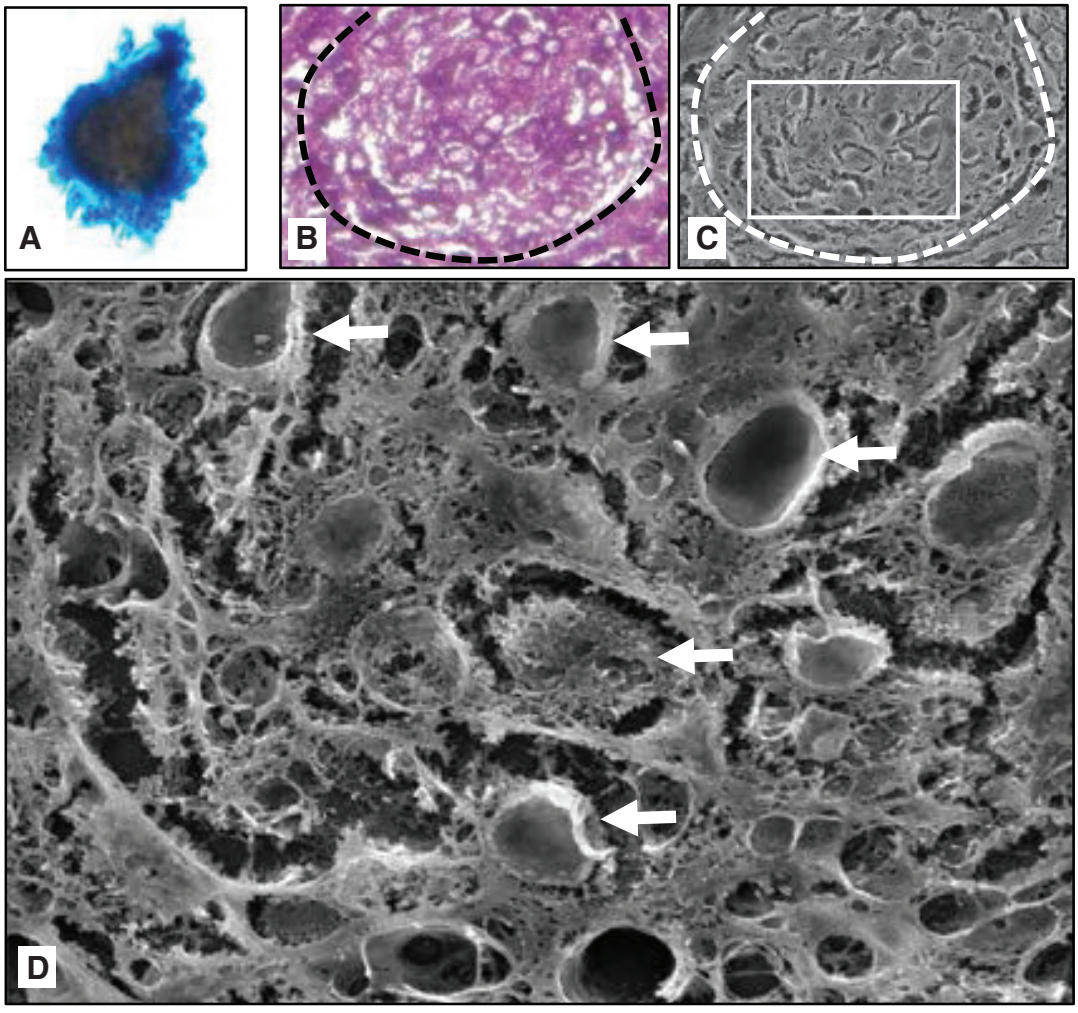

FIG. 3: Morule microstructure

A. Cytology of cellular cluster suggestive of a morule (magnification, x400). The microstructure of morule observed using correlative light and electron microscopy. **B.** Image of morule observed under an optical microscope with hematoxylin-eosin staining. Structures surrounded by a black dotted line indicate a morule (magnification, x2000). **C.** Image of the same morule observed under a scanning electron microscope (magnification, x2000). The region within the white dotted line is magnified in the adjacent panel**. D.** White arrow indicates a cellular nuclear region filled with an amorphous substance, recognised as nuclear clearing under an optical microscope in **B.**

may recapitulate the hair formation observed in embryogenesis.[6] It should be noted that the microstructure of morules of CMV was firstly analysed by CLEM. The nuclei of morular cells were filled with an amorphous substance. This amorphous substance may be a microfilament, as observed by Yamashita *et al*. under transmission electron microscopy in 1992.[7] Yamashita *et al*. showed that the nuclear clearing reacts nonspecifically with various antibodies.[7] At present, it is known that the optically clear nuclei in the morule contain abundant biotin, which is reactivated by the avidin-biotinylated peroxidase complex (ABC) and labeled with streptavidin-biotin (LSAB) method. We also performed the LSAB method for immunohistochemistry, which revealed nonspecific immunostaining in the morular cells with nuclear clearing in all antibodies assessed (data not shown). Therefore, the immunoreactivity of nuclear clearing in morules should not be considered positive when the ABC or LSAB method is used.

CONCLUSION

CMV may be considered as an independent category, for example, "cribriform-morular thyroid carcinoma," and not as one of the variants of PTC because of its distinct clinicopathological characteristics.[2] There is merit in comparing the cytohistology of CMV and other morular-forming tumours and revealing their pathological similarities and differences.[8] The results reported here contribute to our understanding of the clinicopathological features of CMV.

Conflict of interest

The authors declare that they have no competing interests.

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