Childhood thyroid carcinoma with *BRAF*^{T1799A} mutation shows unique pathological features of poor differentiation

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Abstract. The prevalence of *BRAF*^{T1799A} mutation in papillary thyroid carcinomas (PTCs) displays a marked age association: relatively high in adults and exceedingly low in childhood. We report a case of a 12-year-old Japanese female with PTC, the only one case that harbored *BRAF*^{T1799A} mutation in a series of 46 childhood thyroid cancer tissues. On histology, the findings were so atypical that pathologists had repeatedly examined tumor sections to agree on the diagnosis of poorly differentiated follicular carcinoma. Upon molecular investigation, *BRAF*^{T1799A} was detected in DNA extracted from paraffin-embedded material, whereas *TP53* was wild-type. Since *BRAF*^{T1799A} is strictly limited to PTCs, immunohistochemical staining for CD15, a specific marker for papillary carcinoma, was performed to verify the diagnosis. A small tumor area with papillary-like structure was stained positive. These findings strongly suggest that this case is a poorly differentiated carcinoma that arose from PTC and implies a possible association of *BRAF* mutation with dedifferentiated phenotype of PTCs.

Introduction

BRAF^{T1799A} mutation is the most common genetic alteration in adult papillary thyroid carcinomas (PTCs). This mutation is believed to convert *BRAF* into a catalytically active conformation, resulting in constitutive activation of MAP kinase pathway. During the last few years, investigations of the relationship between *BRAF*^{T1799A} mutation and clinicopathological features of thyroid tumors have revealed several salient features such as its exclusive association with PTCs (1-5) and particularly high mutational prevalence in tumors with classical papillary and tall cell morphology (3,6). On the other hand, follicular variant of PTCs has been shown to be mostly *BRAF* wild-type. Moreover, *BRAF* mutation has not been found in normal thyroid tissue, benign tumors or follicular thyroid carcinomas. Thus, *BRAF* genetic analysis using fine needle aspiration biopsy samples is very effective for pre-operative diagnosis of PTCs (7-9). Usefulness of *BRAF* mutational analysis for suspected PTC is further strengthened by the fact that *BRAF* alteration is almost restricted to a hot spot in exon 15: a thymine-to-adenine transversion at nucleotide 1,799 (T1799A), resulting in a valine-to-glutamic acid substitution at amino acid 600 (V600E).

The frequency of *BRAF* mutation strikingly differs between adult and childhood PTCs. In adult patients, *BRAF* mutation is detected in 30 to 60% of cases (1-5), whereas childhood PTCs are usually *BRAF* mutation-free (10,11). Childhood PTCs are rarely diagnosed as an immediately life threatening condition and have a relatively good prognosis compared with adult PTCs. Therefore, we hypothesized that the differences in the prevalence of *BRAF*^{T1799A} mutation between childhood and adult cases may reflect inherent differences in the clinical features of this type of cancer between the two age groups.

We report a case of thyroid cancer in a 12-year-old female patient with an atypical histological pattern. The initial diagnosis of poorly differentiated follicular thyroid carcinoma was subject to change after the molecular analysis of *BRAF*^{T1799A} mutation. Both molecular testing and pathological analysis of this case suggest that *BRAF*^{T1799A} mutation may be associated with dedifferentiation and aggressiveness of PTCs.

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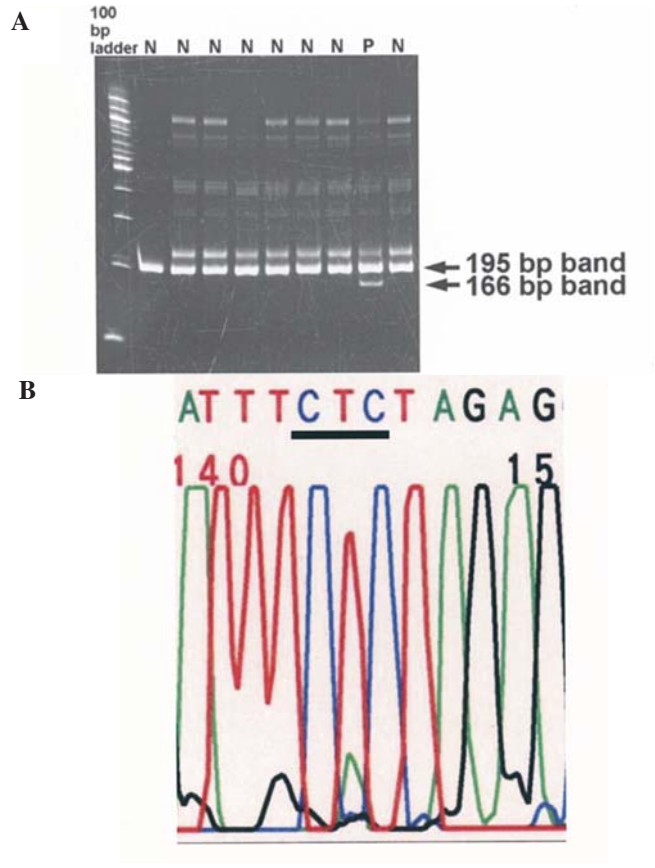

Figure 1. (A) Detection of *BRAF*^{T1799A} mutation by PCR-RFLP method. The 199 bp bands correspond the wild-type allele of *BRAF* gene. The additional 166 bp band corresponds to the mutant allele. (B) Direct sequencing analysis of the portion of *BRAF* exon 15. Underlined is codon 600 with heterozygous point mutation at position 1799 (a T to A transversion).

Materials and methods

Case profile. Twelve-year-old female visited a physician after noticing a nodule in the left side of her neck that gradually enlarged during a 6 month period. There was no relevant family or past medical history of any particular thyroid disease. Physical examination revealed a large firm neck nodule in the left thyroid lobe. Regional lymph nodes were not palpable. Thyroid function blood tests showed euthyroid state. The anti-thyroglobulin antibody and anti-thyroid peroxidase antibody titers were within normal range. Ultrasonography revealed a 6.5-cm left thyroid nodule with cystic region. The right lobe appeared intact. For diagnosis, fine needle aspiration biopsy of the intra-cystic mass region was performed. On cytology, multiple clusters of cells of mostly follicular epithelial origin with small nuclei occasionally accompanied by the clusters containing scattered cells with giant nuclei were found indicative of thyroid cancer.

The surgical treatment including left thyroid lobectomy and ipsilateral neck lymph node dissection was performed at Ito hospital (Tokyo, Japan). Macroscopic examination of the resected thyroid tissue showed a soft encapsulated nodule measuring 6.5 cm, located to the center of the left lobe. The cutting surface of the tumor was a khaki color. The central region of the tumor appeared hemorrhagic or necrotic.

The initial histological diagnosis was follicular thyroid carcinoma with focal undifferentiated pattern and vascular and capsular invasion. Because of the atypical pathological findings and associated uncertainty of diagnosis, the pathological specimen was consulted by thyroid pathologists whose conclusion was a diagnosis of poorly differentiated follicular thyroid carcinoma.

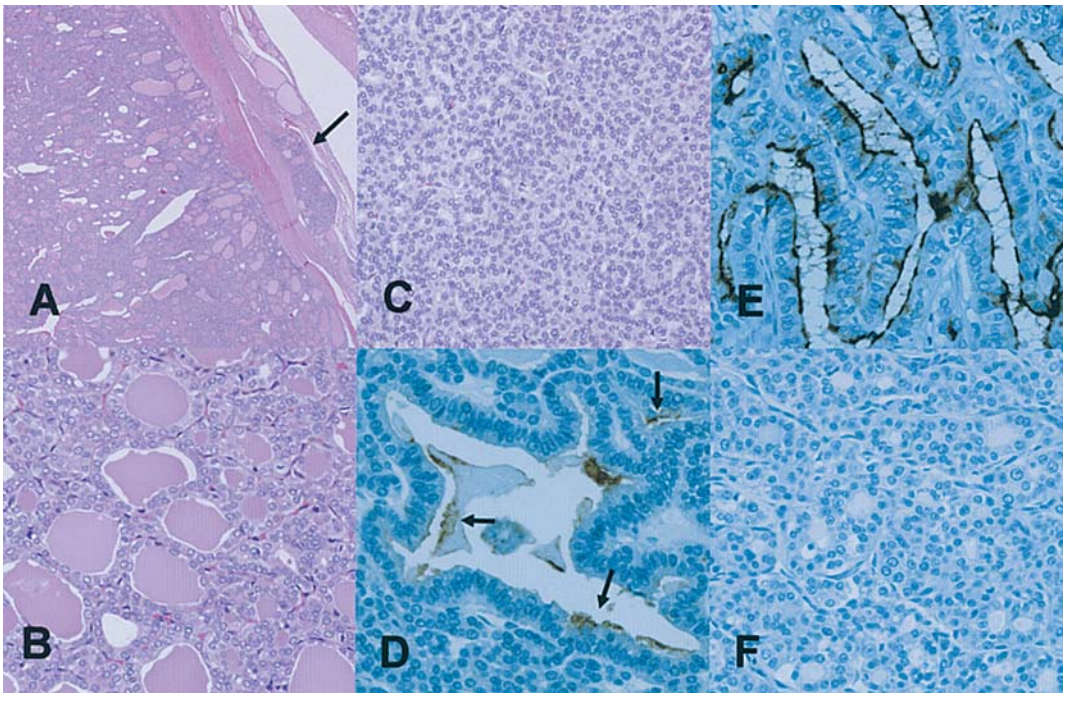

Figure 2. Pathological case findings.(A) Low-power view of hematoxylin-eosin (H&E) staining of tumor. Encapsulated follicular pattern with vascular invasion (indicated by an arrow). (B) High-power view of H.E. staining of follicular component. Round circular nuclei with granular chromatin were observed. (C) H&E staining of solid cellular cluster. This area consists of uniform tumor cells and contains occasional microfollicles without colloid. (D) CD15 staining of the papilloid component. The nuclei of this component show elongation and overlapping. Some cells demonstrate CD15 expression (indicated by arrows). (E) CD15 staining of typical PTC as a positive control. (F) CD15 staining of irrelevant thyroid follicular carcinoma as a negative control.

The patient had no complications after operation. She has been maintained on thyroid hormone (L-Thyroxin, 100 μg/day) suppression therapy since October 1985 until present. During follow-up, no disease relapse such as lymph node metastases and distant metastases was noted in the patient's neck, lungs or bones.

DNA extraction. Genomic DNA was extracted from the mechanically microdissected paraffin-embedded tissue using DEXPAT reagent (Takara, Kyoto, Japan) according to the manufacturer's protocol.

BRAF and TP53 genetic analysis. *BRAF* gene codon 600 status was analyzed by Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and direct sequencing as described in our previous reports (9,10).

TP53 exons 5, 7 and 9 were analyzed by direct sequencing as mutations in these regions have been previously associated with thyroid tumors (12-15). The following primer pairs were used: exon 5, forward (5'-CTGTTCACTTGTGCCCTGAC-3') and reverse (5'-AACCAGCCCTGTCGTCTCTC-3'); exon 7, forward (5'-CATCTTGGGCCTGTGTTATC-3') and reverse (5'-CTGAGTGGGAGCAGTAAGGAG-3'); exon 9, forward (5'-GGTGCAGTTATGCCTCAGATTCAC-3') and reverse (5'-CCCAATTGCAGGTAAAACAGTCAAG-3'). PCR reactions were performed using standard PCR conditions [95˚C for 2 min; followed by 40 cycles of 94˚C for 30 sec, 58˚C (exon 5), 56˚C (exon 7) or 61˚C (exon 9) for 30 sec and 72˚C for 30 sec; then 72˚C for 10 min] with Ex Taq polymerase (Takara Co., Japan).

Immunohistochemistry. CD15 immunostaining was performed to detect the components of thyroid papillary carcinoma as previously described (10). In addition, p53 immunostaining was performed to evaluate the expression pattern of p53. Briefly, the deparaffinized section was blocked with 1% BSA and then incubated overnight at 4˚C with an optimal dilution (0.1 μg/ml) of primary mouse monoclonal antibody against human p53 (Dako, Glostrup, Denmark). The slide was sequentially incubated with biotinylated horse anti-mouse IgG, and the reaction product was visualized using diaminobenzidine treatment.

Results and discussion

Examination of $BRAF^{T1799A}$ mutation using PCR-RFLP method detected only one positive case in a series of 46 childhood thyroid cancer tissues (Fig. 1A). The result was confirmed by direct sequencing analysis (Fig. 1B). We were interested in this case because $BRAF^{T1799A}$ mutation is exceptionally rare in childhood thyroid cancer. Upon medical record investigation, it was noted that the tumor tissue displayed atypical histological features causing difficulty in pathological diagnosis. This case was initially diagnosed by pathologists as poorly differentiated follicular thyroid carcinoma, prior to *BRAF* genetic analysis.

Since previous studies have demonstrated that the *BRAF* mutation is exclusively restricted to PTCs and is not detected in follicular thyroid adenoma and carcinoma, hematoxylin and eosin-stained specimens were carefully re-examined by experienced international pathologists. Most part of the tumor consisted of follicular (Fig. 2A and B) and solid (Fig. 2C) structures composed of cells without typical nuclear features associated with PTCs such as grooving and ground grass appearance. These findings reaffirmed the initial diagnosis of poorly differentiated follicular carcinoma. Then we performed immunohistochemical staining for CD15 to further clarify the histological type of this case, because a previous study reported that CD15 expression was specific to papillary thyroid carcinoma (16). Some cells in papilloid components were stained positive (Fig. 2D), suggesting that this case was poorly differentiated carcinoma that arose from PTC.

Since *TP53* mutations are thought to play an important role in dedifferentiation of thyroid cells (12-15), we performed *TP53* mutational analysis and p53 immunohistochemistry. In the case under consideration, we did not detect *TP53* gene abnormality and nuclear p53 overexpression (data not shown). These data suggest that $BRAF^{T1799A}$ mutation may be a major oncogenic event in the childhood tumor with a unique poorly differentiated morphology.

Although it is still controversial, $BRAF^{T1799A}$ mutation have been reported to be associated with advanced clinical features of PTCs in several reports (2,17,18), especially in the most recent large-scale study (19). The bovine thyroglobulin promoter-driven $BRAF^{T1799A}$ transgenic mice developed invasive PTC-like tumors within three months after birth (20). Although this model may incompletely resemble human PTCs because of the high serum TSH level found in the animals, it is interesting that the tumors displayed manifestations of poor differentiation in parallel with downregulation of thyroid-specific gene expression. Similar findings were not reported in RET/PTC transgenic mice (21), suggesting that $BRAF^{T1799A}$ mutation may promote dedifferentiation and invasion of PTCs. Mitsutake *et al* demonstrated that $BRAF^{T1799A}$ mutation induced chromosomal instability in thyroid cells *in vitro* (22). Presumably, the $BRAF^{T1799A}$ oncogenic mutation may foster other genetic alterations which may eventually contribute to tumor dedifferentiation and invasiveness.

In conclusion, we describe a case of childhood poorly differentiated PTC with $BRAF^{T1799A}$ mutation but retaining wild-type *TP53*. This case was initially diagnosed as poorly differentiated follicular carcinoma because of atypical morphology. However, our molecular analysis assisted in making a final diagnosis, supporting an efficacy of *BRAF* mutational analysis in thyroid tumors with atypical features. This case taught us that $BRAF^{T1799A}$ is a potential marker of PTCs for not only pre- but also post-operative diagnosis in difficult cases. When we vacillate between papillary carcinoma and follicular carcinoma, *BRAF* gene analysis could be an important complementary method for pathological diagnosis. Since childhood PTC with $BRAF^{T1799A}$ mutation is an extremely rare type of thyroid cancer, purposeful collection of such cases and careful examination are necessary to clarify its characteristics; in particular, association between $BRAF^{T1799A}$ mutation and clinicopathological features such as dedifferentiation and aggressiveness.

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