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Immunohistochemical and Molecular Analyses Focusing on Mesenchymal Cells in Papillary Thyroid Carcinoma with Desmoid-Type Fibromatosis

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Abstract
Objective: This study was designed to evaluate the prevalence of *CTNNB1* (β-catenin) mutations in cases of papillary thyroid carcinoma with desmoid-type fibromatosis (PTC-DTF) expressing aberrant nuclear and cytoplasmic immunoreactivity for β-catenin. ***Methods:*** Eight cases of PTC-DTF were available for this study. Immunohistochemistry for β-catenin and $BRAF^{V600E}$ was performed. *CTNNB1* and *BRAFV600E* mutations were also evaluated by direct sequencing. ***Results:*** For β-catenin, although we could demonstrate aberrant nuclear and cytoplasmic immunoreactivity in DTF components in all cases, suggesting activated Wnt signaling, direct sequencing revealed a missense mutation, c.121A>G (p.T41A), in exon 3 in only one case, and no mutations in exons 3, 4, and 5 in the other cases. In the $BRAF^{V600E}$ analyses, immunohistochemistry revealed positive staining in the carcinoma cells but not DTF components of all cases. These findings were subsequently validated by direct sequencing. ***Conclusion:*** This study suggests the significance of the *BRAFV600E* mutation and activation of Wnt signaling pathway in the carcinoma cells and DTF components, respectively. We believe that the *CTNNB1* mutations are not the major factor behind β-catenin translocation indicating Wnt pathway activation. Further study is required to evaluate whether molecular abnormalities other than the *CTNNB1* mutation cause activation of Wnt signaling in DTF components of PTC-DTF.

Introduction

Papillary thyroid carcinoma (PTC) with fibromatosis/nodular fasciitis-like stroma is a rare variant that presents with extensive proliferation of mesenchymal cells such as fibroblasts and myofibroblasts in the stroma [1]. Because aberrant nuclear and cytoplasmic immunoreactivity for β-catenin is observed in the mesenchymal components, it has been suggested that activated Wnt signaling is involved in the stromal overgrowth of this variant [2].

β-Catenin is a protein encoded by *CTNNB1* and appears to be a key downstream effector of the Wnt pathway

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Table 1. Detail of analyses for genetic alterations

Gene	Exon	Primer sequence	Annealing temperature, °C	Amplicon size, bp	Number of cycles
BRAF	exon 15	5′-TGCTGAACTGTGGATAGTGAGTG-3′ 5′-CAACTGCATGTTTCAGCATCT-3′	62	173	45
CTNNB1	exon 5	5′-TTCAGAATGTCTACCCAATACCA-3′ 5′CATCTGAGGAGAACGCATGA-3′	60	152	40
CTNNB1	exon 5	5′TCATGCGTTCTCCTCAGATG-3′ 5′ATTTTCACCAGGGCAGGAAT-3′	60	166	40
CTNNB1	exon 4	5′TGCTGAACTGTGGATAGTGAGTG-3′ 5′CAACTGCATGTTTCAGCATCT-3′	63	150	35
CTNNB1	exon 4	5′ACTCGAGCTCAGAGGGTACG-3′ 5′TGTGGCAAGTTCTGCATCAT-3′	60	186	40
CTNNB1	exon 3	5′-TGTGGCAAGTTCTGCATCAT-3′ 5′-CAATGGGTCATATCACAGATTCTT-3′	63	118	40

for regulating cell growth/survival [3]. This pathway is activated by genetic mutations including *CTNNB1* exon 3 and adenomatous polyposis coli (*APC*) protein, that stabilize the β-catenin protein which accumulates in the cytoplasm and then translocates to the nucleus [4]. It then binds to the T cell factor/lymphoid enhancer factor to activate genes, such as cyclin D1, and contributes to the oncogenesis of various human tumors including desmoid-type fibromatosis (DTF) of soft tissue [5].

In thyroid cancer, although genetic mutations of *CTNNB1* or *APC* are not observed in conventional PTC, they have been reported in peculiar subtypes such as the cribriform variant of PTC, with or without familial adenomatous polyposis and anaplastic carcinoma [6]. Recently, Rebecchini et al. [2] reported a mutation in exon 3 of *CTNNB1* in the mesenchymal components of two cases of PTC with fibromatosis/nodular fasciitis-like stroma. The involvement of the activated Wnt/β-catenin pathway has not been observed in nodular fasciitis, so they proposed a more suitable term for this variant: PTC with desmoid-type fibromatosis (PTC-DTF) [2]. This study was designed to evaluate the prevalence of *CTNNB1* mutations in more cases of PTC-DTF exhibiting aberrant nuclear and cytoplasmic immunoreactivity for β-catenin.

Materials and Methods

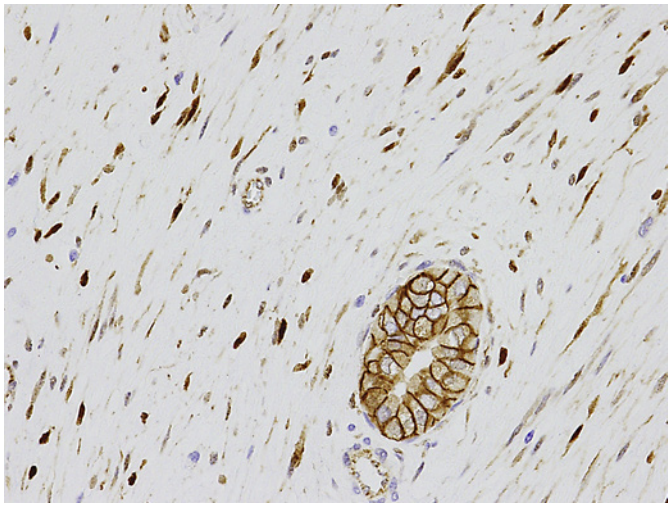
The study protocol was reviewed and approved by the Institutional Review Board of the Kuma Hospital (20180208–2). Eight PTC-DTF cases that were resected in Kuma Hospital between January 2007 and December 2016 were extracted for this study. They included 5 women and 3 men with a mean age of 44.8 years (range 29–68 years). A diagnosis of PTC-DTF was made on the basis of extensive proliferation of fibroblasts and myofibroblasts in the stroma of PTC. Clinical data were obtained from patients' medical records at the Kuma Hospital.

Formalin-fixed paraffin-embedded (FFPE) tissue sections were used for immunohistochemistry (IHC) and mutation analyses for both β-catenin and $BRAF^{V600E}$. IHC for β-catenin (1:400 dilution, 14, BD Biosciences, Franklin Lakes, NJ, USA) was performed using the automated Leica Bond-Max system with a Bond Refine detection kit (Leica Microsystems, Wetzlar, Germany), according to the manufacturer's recommendations. IHC for $BRAF^{V600E}$ (1:100 dilution, VE1, Spring Bioscience, Pleasanton, CA, USA) was performed manually.

For gene mutation analyses, PTC and DTF components were microdissected from each FFPE section and transferred separately into tubes. DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and screened for genetic alterations. Details of the PCR conditions for detecting genetic alterations are summarized in Table 1. Amplicon aliquots were treated with ExoSAP-IT (USB Corp., Cleveland, OH, USA) and sequenced on an ABI Prism 3130 automated capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA) using the BigDye terminator cycle sequencing ready reaction kit v3.1 (Applied Biosystems).

Results

The mean proportions of DTF areas in PTC ranged from 20 to 95%. Distribution of the DTF areas was classified into 3 types: central (3 cases), mixed (3 cases), and diffuse (2 cases). DTF areas were composed mainly of dense collagenous connective tissue with less cellular fibroblastic proliferation. The nuclei of the fibroblasts were slender. Carcinoma cells were paucicellular in DTF areas and the nuclei showed typical characteristics of PTC. In non-DTF areas, PTC histology was the conventional type. Two cases



Color version available online

Fig. 1. Immunohistochemistry for β-catenin. Fibroblasts show nuclear and cytoplasmic positivity for β-catenin. Carcinoma cells show membranous positivity for β-catenin. ×200.

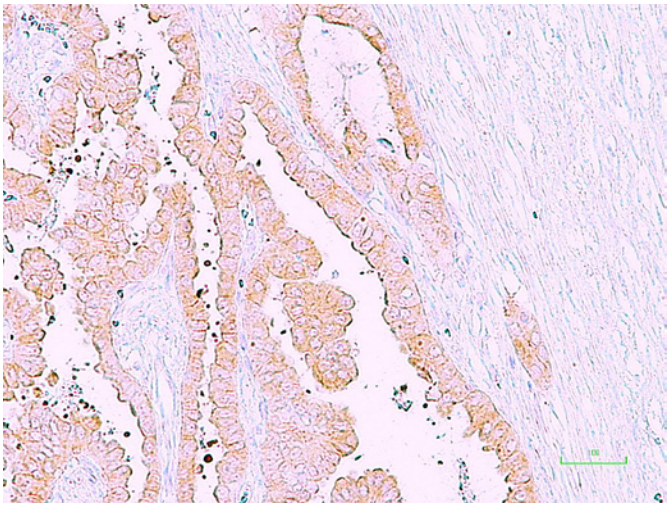


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Fig. 2. Immunohistochemistry for $BRAF^{V600E}$. Carcinoma cells are positive for $BRAF^{V600E}$. Fibroblasts are negative for $BRAF^{V600E}$. ×200.

Table 2. Comparisons of clinicopathological profiles of PTC with desmoid-type fibromatosis between mutated and wild-type *CTNNB1* exon 3

	CTNNB1 exon 3	
	mutated (n = 1)	wild-type (n = 7)
Age, years	20	34–68 (47.1)
Male/female	0/1	3/4
Serum thyroglobulin, ng/mL	189	3.1–85.4 (44.4)
Free thyroxine, ng/mL	0.98	0.82–1.13 (1.00)
Tumor size, mm	79	16–73 (36.4)
Proportion of fibromatosis, %	80	20–95 (71.4)
Type of fibromatosis distribution		
Central	1	2
Mixed	0	3
Diffuse	0	2
Association with chronic thyroiditis	0	2
Nodal metastasis	1/1	6/6
Presence of DTF	0/1	2/6
Distant metastasis[a]	0	0
Immunohistochemistry		
Aberrant β-catenin in DTF	1	7
$BRAF^{V600E}$ in PTC	1	7
BRAF ^{V600E}	1	6/6

Values express n or range (mean). DTF, desmoid-type fibromatosis; PTC, papillary thyroid carcinoma.

[a] During surgery and during follow-up (1–10 years).

were associated with chronic thyroiditis. Nodal metastasis was demonstrated in all 7 cases with lymph node resection. In 2 cases, DTF components were observed in some nodal metastatic lesions. Distant metastasis was not detected during surgery or follow-up in all cases.

IHC for β-catenin revealed aberrant nuclear and cytoplasmic immunoreactivity in the mesenchymal cells of the DTF components. However, in all cases, the carcinoma cells exhibited intact membranous staining (Fig. 1). In contrast, immunopositivity for $BRAF^{V600E}$ was evident in the cytoplasm of carcinoma cells but not mesenchymal cells (Fig. 2). The mutation analyses demonstrated no mutations in exons 3, 4, and 5, but one case of a missense mutation, c.121A>G (p.T41A), in exon 3 of *CTNNB1* in the DTF component. We could extract sufficient DNA for analysis from the FFPE sections and the $BRAF^{V600E}$ mutation was evident in the PTC components from all 7 cases. Comparisons of clinicopathological profiles of cases between mutated and wild-type *CTNNB1* exon 3 are summarized in Table 2.

Discussion

Microscopic and IHC findings of DTF and the stromal areas of PTC-DTF are quite similar [2, 7]. They are composed of dense collagenous connective tissue with fibroblastic proliferation. The fibroblasts are hypocellular and the nuclei are slender. Small-sized vessels with thick walls

are scattered in the stroma. Immunohistochemically, the fibroblasts show nuclear and cytoplasmic positivity for β-catenin. *CTNNB1* mutations have been detected in up to 85.0% of sporadic DTF cases [8], resulting in the stabilization and translocation of β-catenin into the nucleus where it acts as a transcription factor. It was also demonstrated that the 5-year recurrence-free survival rate of DTF patients with these mutations is significantly worse than that of patients with wild-type *CTNNB1* [9].

A recent study demonstrated a missense mutation, c.133T>C (p.S45P), in exon 3 of *CTNNB1* in the DTF components of two cases of PTC-DTF [2]. Our study also detected a missense mutation in exon 3 of *CTNNB1*, i.e., c.121A>G (p.T41A), in the DTF components from 1 out of 8 cases of PTC-DTF showing aberrant nuclear and cytoplasmic immunoreactivity of β-catenin. Further analyses of exons 4 and 5 of *CTNNB1* could not find any mutations in our series. Similarly, the β-catenin mutations were detected in only 26% of gastric carcinomas that showed β-catenin nuclear staining [10]. We first confirmed the missense mutation of *CTNNB1* in the DTF components reported by Rebecchini et al. [2], but the mutation we found was different. In this study, we demonstrated that β-catenin nuclear staining and the *CTNNB1* mutations are not always correlated. We believe that the *CTNNB1* mutations are not the major factor for β-catenin translocation indicating Wnt pathway activation. Involvement of a molecular abnormality other than the *CTNNB1* mutation may cause aberrant β-catenin expression in the mesenchymal cells of PTC-DTF.

In the $BRAF^{V600E}$ analyses, IHC revealed positive staining in the PTC but not the DTF components of all cases. This was subsequently validated by direct sequencing as well as a previous study by Rebecchini et al. [2]. Although an association between $BRAF^{V600E}$ mutation and recurrence, nodal metastasis, extrathyroidal extension, and advanced stage is suggested in PTC [11], both recurrence and distant metastasis were not evident in our cases during the follow-up period. Therefore, our study suggests the significance of the $BRAF^{V600E}$ mutation and activation of the Wnt signaling pathway in the carcinoma cells and DTF components, respectively. However, the relationship between the BRAFV600E mutation in cancer cells and aberrant expression of β-catenin in DTF components is still unclear. Because the $BRAF^{V600E}$ and *CTNNB1* mutations are not directly related to their respective pathways, the coincidence seems to be independent. Further studies are required to evaluate the presence of molecular abnormalities other than the *CTNNB1* mutation that may activate Wnt signaling in the DTF components of PTC-DTF.

Disclosure Statement

The authors declare no conflicts of interest.

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