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Genetic profiling of thymic carcinoma using targeted next-generation sequencing

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ABSTRACT

Objectives: Thymic carcinoma is a rare mediastinal neoplasm and little is known about its tumorigenesis. There is no effective treatment except for complete resection, and the prognosis of advanced cases is poor. To identify the mutations associated with tumorigenesis, we analyzed genetic profile of thymic carcinoma using targeted next-generation sequencing.
Materials and methods: We sequenced about 409 cancer-related genes in 12 thymic squamous cell carcinoma tissues including 10 tumor/normal tissue pairs using Ion AmpliSeq Cancer Panel and Ion PGM Sequencer. We filtered the mutations with Ingenuity Variant Analysis, SIFT, PolyPhen-2, and PROVEAN.
Results and conclusion: Twenty-five candidate mutations in 24 genes were identified, including five tyrosine kinase genes (*KIT*, *DDR2*, *PDGFRA*, *ROS1*, *IGF1R*). There was no recurrent mutation among the samples studied. The *KIT* exon 11 deletion mutation in 1 patient was an activating mutation and may be an oncogenic driver mutation. Genetic profiling of thymic carcinoma using targeted next-generation sequencing was performed. The mutation status of thymic squamous cell carcinoma is highly heterogeneous.

1. Introduction

Thymic carcinoma is a rare tumor of thymic epithelial origin. Thymic carcinomas are characterized by their histologic variability, often resembling tumors seen in other organ systems. This morphologic variation coupled with their rarity has prevented large-scale research of this tumor and little is known about their etiology, biologic behavior or adequate treatment [1]. There is no effective treatment except for complete resection, and the prognosis of the advanced and recurrent cases are poor [2–5].

Up to now, several efforts have been made to identify the molecular pathways underlying the tumorigenesis of thymic carcinoma in order to develop treatment strategies [1]. But, each genomic analysis has covered only a few recurrently genes such as *EGFR* [6–8], *Her2* [9,10], *Kit* [7,8,11], *Kras* [8,12] and *p53* [13,14].

Recently, next-generation sequencing (NGS) has been developed. NGS has enabled whole-exome and targeted sequencing of tumors for causative mutations, allowing for more accurate targeted therapies. Already, the combination of massively parallel sequencing and selective capture approaches has facilitated efficient simultaneous genetic analysis of a large number of candidate genes [15]. This method is effective and ideal for rare tumors like thymic carcinoma to analyze the molecular mechanism of tumorigenesis.

To identify the mutations associated with tumorigenesis of thymic carcinoma, we sequenced all the exons of 409 cancer-related genes using massively parallel sequencing using 12 thymic carcinomas tissues.

2. Materials and methods

2.1. Patients

This study included 12 thymic carcinoma patients who underwent surgery at the Nagoya City University Hospital between 1992 and 2013. Patients characteristics are shown in Table 1. Briefly, the 12 patients ranged in age from 48 to 75 years, with a mean age of 63. They consisted of two females and 10 males. All of them were diagnosed as squamous cell carcinomas (SCC). Sometimes the diagnosis of the thymic carcinoma is difficult. The diagnosis of the thymic carcinoma was held by several specific immunohistochemistry, including CD5, c-KIT and bcl-2. Fig. 1 is

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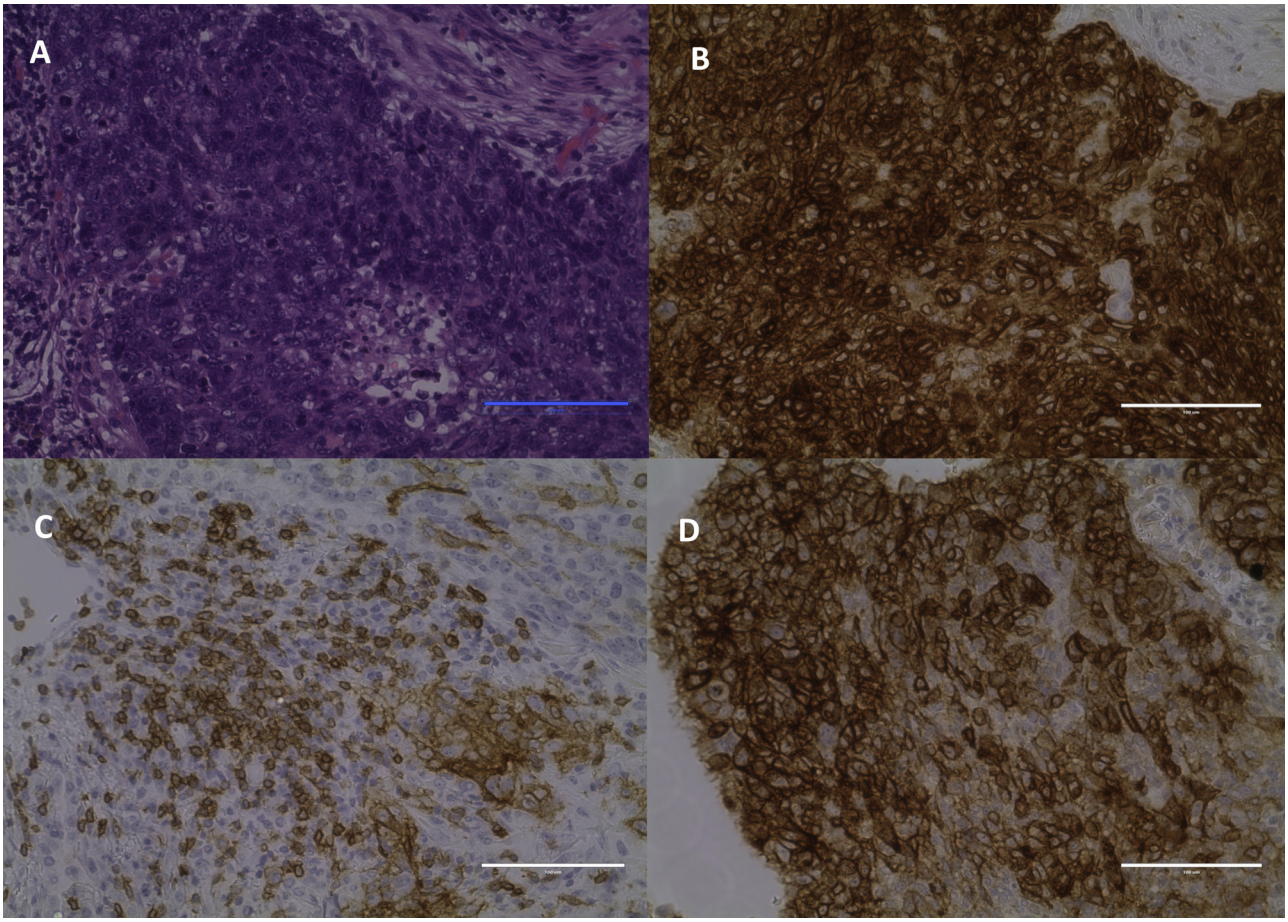

Fig. 1. Immunohistochemistry of thymic carcinoma patient (Patient 9). (A) Hematoxylin–Eosin (400×), (B) bcl-2 (400×), (C) CD5 (400×), (D) c-KIT (400×).

the CD5, c-KIT and bcl-2 immunohistochemistry of the Patient 9. We used the most recent revision of the World Health Organization histologic TNM classification and stage grouping of thymic epithelial tumors in 2004 (World Health Organization classification of tumors. Pathology and genetics. International Agency for Research on Cancer Press, 2004, 146–151) and the Masaoka staging system [16]. In 10 patients, matched normal thymic tissue was available for analysis. The present study was approved by the Institutional Review Board of Nagoya City University Hospital, and a written consent was obtained from all the patients.

Table 1
The clinical and pathological characteristics of the 12 thymic carcinoma patients.

Patient	Age	Sex	Pathological subtype	Masaoka stage	WHO-stage
1	73	Male	SCC	II	II
2	49	Female	SCC	III	III
3	61	Male	SCC	IVb	IV
4	71	Male	SCC	III	III
5	62	Male	SCC	II	II
6	76	Male	SCC	IVb	IV
7	61	Male	SCC	III	III
8	48	Female	SCC	IVb	III
9	65	Male	SCC	III	III
10	54	Male	SCC	IVa	IV
11	66	Male	SCC	IVb	IV
12	75	Male	SCC	II	II

SCC, squamous cell carcinoma.

2.2. DNA preparation

All tumor samples were immediately frozen and stored at −80 °C until assayed. Tumor samples were obtained from 12 patients with thymic SCC. Germline DNA was obtained from adjacent, histologically normal tissues resected at the time of surgery ($n = 8$) or from peripheral blood ($n = 2$). DNA was extracted from fresh frozen tissue using QIAamp DNA Mini Kit (QIAGEN; Hilden, Germany) according to the manufacturers' instructions.

2.3. Ion Torrent next-generation sequencing

The Ion AmpliSeq Comprehensive Cancer Panel (Life Technologies Corporation; Carlsbad, CA, USA) was used to generate target amplicon libraries. Briefly, 40 ng of DNA was amplified by polymerase chain reaction (PCR) with the Ion AmpliSeq Comprehensive Cancer Panel Primer Pool and Ion AmpliSeq Library Kit 2.0 with the protocol recommended by the manufacturer. The multiplexed library targets all the exons of 409 tumor suppressor genes and oncogenes (Table 2), with nearby 16,000 primer pairs. Library concentration and amplicon size was determined with an Agilent High Sensitivity DNA Kit (Agilent Technologies, Inc, Waldbronn, Germany). Samples were multiplexed for emulsion PCR and sequencing on an Ion PGM Sequencer (Life Technologies Corporation). Sequencing was performed using the Ion PGM 200 Sequencing Kit according to the manufacturer's instructions on Ion 318 Chips.