

Rare frequency of gene variation and survival analysis in thymic epithelial tumors

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Objective: Thymic epithelial tumor (TET) is a rare mediastinal neoplasm and little is known about its genetic variability and prognostic factors. This study investigated the genetic variability and prognostic factors of TET.

Patients and methods: We sequenced 22 cancer-related hotspot genes in TET tissues and matched normal tissues using Ampliseq Ion Torrent next-generation technology. Overall survival was evaluated using Kaplan–Meier methods and compared with log-rank tests.

Results: A histological analysis of 52 patients with a median age of 52 years showed 15 patients (28.8%) with thymic carcinoma, five with type A thymoma (9.6%), eight with type AB (15.4%), six with type B1 (11.5%), nine with type B2 (17.3%), and nine with type B3 thymoma (17.3%). Three gene mutations were identified, including two with *PIK3CA* mutation and one with *EGFR* mutation. The three patients with mutant genes included two cases of thymoma (one with *EGFR* and the other with *PIK3CA* mutation) in addition to a case of thymic carcinoma (*PIK3CA* mutation). The 5-year survival rates were 77.7% in all patients. The 5-year survival rates were 93.3%, 90.0%, 76.9%, and 22.9% corresponding to Masaoka stages I, II, III, and IV ($P < 0.001$). The 5-year survival rates were 100%, 100%, 83.3%, 88.9%, 65.6%, and 60.9% in the histological subtypes of A, AB, B1, B2, and B3 thymomas, and thymic carcinoma, respectively ($P = 0.012$).

Conclusion: Hotspot gene mutations are rare in TET. *PIK3CA* and *EGFR* mutations represent candidate driver genes and treatment targets in TET. Masaoka stage and histological subtypes predict the survival of TET.

Keywords: thymic epithelial tumors, gene mutation, prognosis

Introduction

Thymic epithelial tumor (TET) is a relatively uncommon tumor of thymic epithelial origin.¹ Previous studies demonstrated that radical surgery is the only effective intervention for long-term survival.^{2,3} However, local recurrence or distant metastasis may occur in some patients even after complete resection. There is no effective treatment for advanced and recurrent disease and the prognosis is poor.⁴

Several studies with limited number of patients have shown that targeted treatment may be effective in specific cases.^{5–7} Partial efforts have been made to identify the driver genes of TET in order to develop treatment strategies.^{8,9} Most of the studies focus on single driver genes, such as *EGFR*, *HER2*, *Kit*, and *KRAS*.^{10–14} Few studies evaluated the gene status in TET with high-throughput technology.¹⁵

Several studies have demonstrated that Masaoka stage was an important prognostic factor for TET. However, the role of other factors, including histological subtypes and myasthenia gravis, remains controversial.^{16–17}

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In the present study, we identified mutations associated with tumorigenesis using next-generation sequencing (NGS) of 22 hotspot genes in a series of 52 TET patients. Furthermore, we evaluated the survival of 52 TET patients to investigate the prognostic factors.

Patients and methods

Patients

Fifty-two patients with pathologically confirmed TET, who underwent diagnosis between January 2007 and December 2012, were identified in Zhejiang Cancer Hospital. Two pathologists blinded to patients' clinical pathology reviewed the samples. The patients were clinically staged according to the Masaoka–Koga system.¹⁸ The histologic types were based on the 2004 World Health Organization classification.¹⁹ The histologic subtypes included type A, AB, B1, B2, B3, and thymic carcinomas. The study was approved by the Ethics Committee of Zhejiang Cancer Hospital and all patients provided written informed consent for biomarker analysis and to have their data used in this study.

NGS detection methods

Microscopy was used to ensure that the tumor tissues analyzed had more than 20% tumor contents. Tumor DNA was isolated from formalin-fixed and paraffin-embedded sections using the QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany), following manufacturer's instructions. Ten nanograms of DNA were used to prepare the bar-coded libraries with the Ion AmpliSeq™ Library kit 2.0 (Thermo Fisher Scientific, Waltham, MA, USA). The panel was used to analyze 1,800 mutational hotspots and targeted regions in 22 genes: *EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *AKT1*, *ERBB2*, *PTEN*, *NRAS*, *STK11*, *MAP2K1*, *ALK*, *DDR2*, *CTNBN1*, *MET*, *TP53*, *SMAD4*, *FBXW7*, *FGFR3*, *NOTCH1*, *ERBB4*, *FGFR1*, and *FGFR2*. Template preparation and enrichment were performed with the Ion OneTouch™ two System (Thermo Fisher Scientific). Finally, sequencing was carried out using Ion 316™ chips on the ion personal genome machine (Ion PGM) System (PGM™, Thermo Fisher Scientific) and with the Ion PGM™ Sequencing 200 kit v2. Alignment to the hg19 human reference genome and variant calling were performed by the Torrent Suite Software version 4.0.2 (Thermo Fisher Scientific). Alignments were visually checked with the Integrative Genomics Viewer (IGV) version 2.3.34.^{20,21} DNA from normal tissue from all samples was also sequenced to check the germline status of the variants.

Statistical analysis

Survival curves were calculated using the Kaplan–Meier method from the start of diagnosis until death or last

follow-up. Statistical analysis was performed using the SPSS 18 software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. The median follow-up period was 65 months (36–118 months) and the last follow-up time was March 31, 2015.

Results

Clinical presentation

Totally, 52 patients, including 20 males and 32 females, with pathologically confirmed TET were enrolled. The clinicopathological characteristics are listed in Table 1. The median age at diagnosis was 52 years (range, 18–71 years). Eleven patients were current or ever smokers and 41 never-smokers. Histologically, there were five patients with type A thymoma (9.6%) (Figure 1A), eight with type AB (15.4%) (Figure 1B), six with type B1 (11.5%) (Figure 1C), nine with type B2 (17.3%) (Figure 1D), nine with type B3 thymoma (17.3%) (Figure 1E), and 15 with thymic carcinoma (28.8%) (Figure 1F). Pathologic stages at diagnosis included: 18 patients with stage I, eleven patients with stage II, 13 patients with stage III, and ten patients with stage IV disease. Totally, four patients were identified as having extrathymic malignancies in addition to thymoma (three patients) and thymic carcinoma (one patient). The incidence of extrathymic malignancies in patients with thymoma was 8.1% for thymoma and 6.7% for thymic carcinoma. The four extrathymic malignancies included lung cancer ($n=1$),

Table 1 Clinical characteristics of the study population

	Number
Sex	
Male	20
Female	32
Age (years)	
Median	52
Range	18–71
<50	21
≥50	31
Smoking status	
Never	41
Former/current	11
Histology ^a	
Type A	5
Type AB	8
Type B1	6
Type B2	9
Type B3	9
Type C	15
Masaoka stage at diagnosis	
I	18
II	11
III	13
IV	10

Note: ^aHistology type is thymoma or thymic carcinoma.

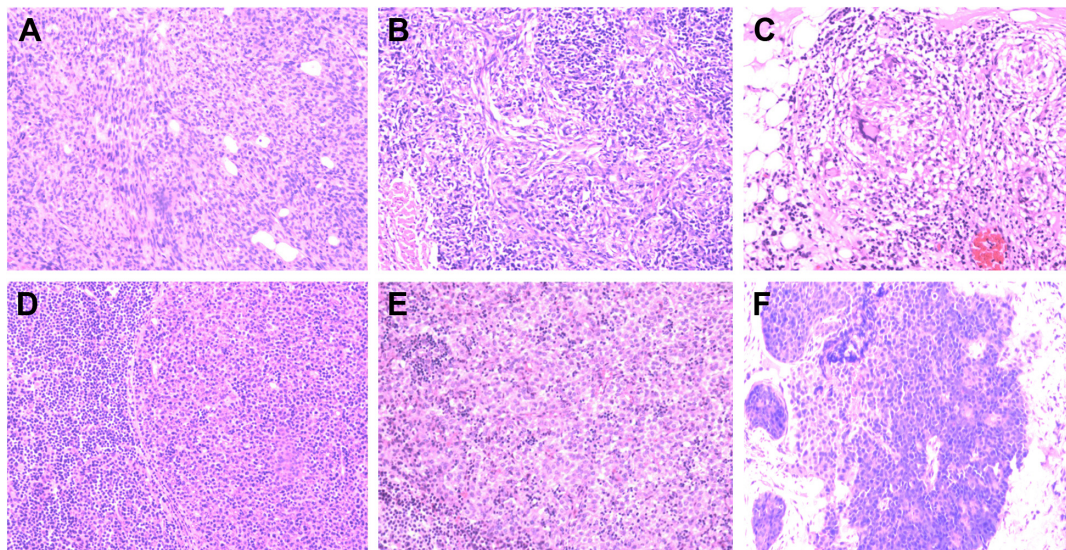


Figure 1 H and E stained images of the different subtypes of thymic epithelial tumors ($\times 40$).

Notes: (A) Type A, (B) type AB, (C) type B1, (D) type B2, (E) type B3, (F) type C.

Abbreviation: H and E, hematoxylin and eosin.

hepatocellular carcinoma (n=1), breast carcinoma (n=1), and colon cancer (n=1).

Molecular analyses

NGS analyses were performed on all the 52 cases. One patient carried an *EGFR* mutation (E746_750de), and two cases harbored *PIK3CA* mutation (both E545Q) (Table 2). No molecular aberrations were found in the other 49 patients.

Treatment

Forty-six patients underwent surgical resection, including 40 with complete resection and six with incomplete resection. The surgical approach included sternotomy (n=25), thoracotomy (n=14), and video-assisted thoracoscopic surgery (n=7). Fifteen patients were treated with radiation therapy (dose range: 40–60 Gy). Twenty-three patients received chemotherapy (six with only chemotherapy, 17 with radiotherapy and chemotherapy).

Survival analyses

The disease-free survival for the 46 patients with surgery was 57.8%. Twenty-one patients received first-line

chemotherapy with median progression-free survival of 8.2 months (95% confidence interval [CI]: 5.1–10.3).

The 5-year survival rate was 77.7% in all patients. The 5-year survival rates in the subtypes A, AB, B1, B2, and B3 thymomas, and thymic carcinoma were 100%, 100%, 83.3%, 88.9%, 65.6%, and 60.9%, respectively ($P=0.012$; Figure 2). The 5-year survival rates were 93.3%, 90.0%, 76.9%, and 22.9% by Masaoka stage I, stage II, stage III, and stage IV ($P<0.001$; Figure 3), respectively.

No significant effects of age ($P=0.739$), sex ($P=0.226$), or myasthenia gravis ($P=0.832$) on overall survival were observed. Additionally, no survival difference was found between patients with and without gene mutation ($P=0.352$; Figure 4).

Discussion

Our study showed that only three samples with two mutation types were found in 52 TET patients. Two cases of *PIK3CA* mutation and one with *EGFR* mutation were identified. To the best of our knowledge, our study represents the largest number of patients with NGS detection in TET. In addition, the Masaoka stage and histological subtypes were confirmed as prognostic factors in our cohort.

Table 2 Clinical characteristics in gene alteration patients

Case	Sex/age (years)	Smoking history	Histology ^a	Gene type	Treatment	OS/months
1	Female/48	Yes	B2	<i>PIK3CA</i>	Surgery	85+
2	Male/55	No	C	<i>PIK3CA</i>	Surgery + radiotherapy	46
3	Female/46	No	B3	<i>EGFR</i>	Surgery	45

Note: ^aHistology type is thymoma or thymic carcinoma.

Abbreviation: OS; overall survival.

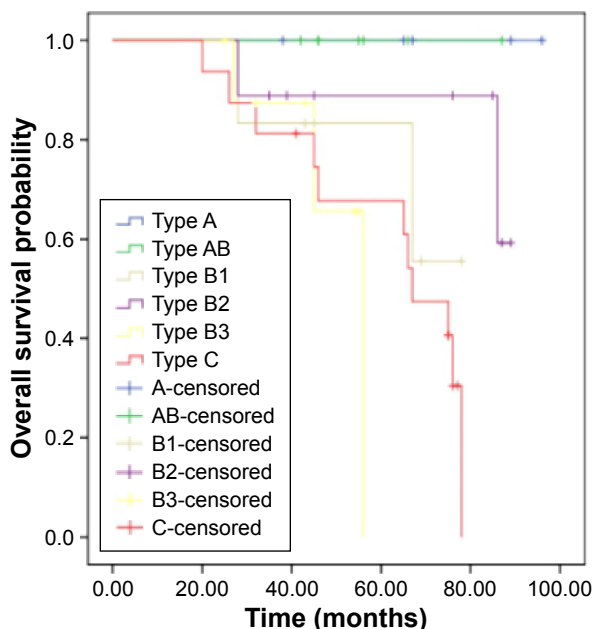


Figure 2 Kaplan-Meier curve of overall survival of different histological subtypes ($P=0.012$).

Thymomas are defined as neoplasms arising from or exhibiting differentiation toward thymic epithelial cells, usually with a variable component of nonneoplastic lymphocytes. Thymic carcinomas are malignant epithelial tumors with overt cytologic atypia, almost invariably invasiveness and lack the

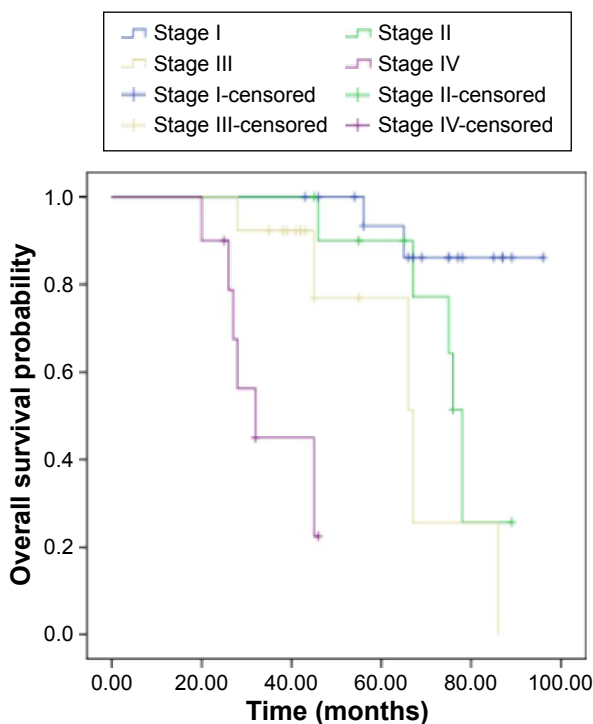


Figure 3 Kaplan-Meier curve of overall survival of different Masaoka stage ($P<0.001$).

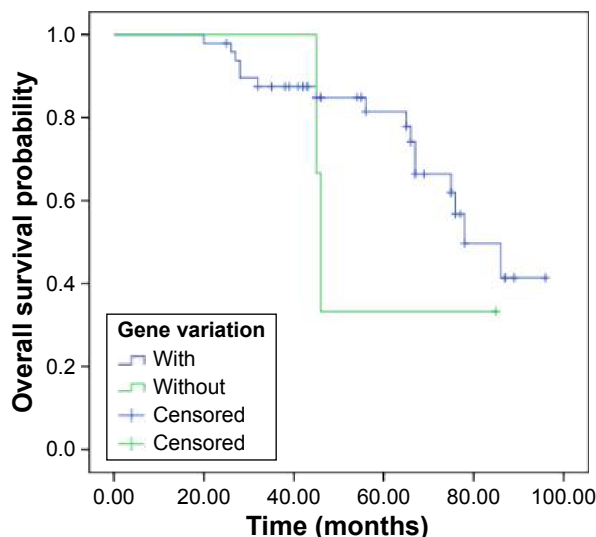


Figure 4 Kaplan-Meier curve of overall survival in patients with ($n=3$) and without ($n=49$) gene variation ($P=0.352$).

organotypic features of thymomas. From a histological point of view, some gene variations commonly seen in epithelial origin tumors may be found in TET. *PIK3CA* pathways play an important role in carcinoma proliferation and metastasis. The frequency of *PIK3CA* mutations is ~2%–5% in other solid carcinomas.^{22,23} Data pertaining to *PIK3CA* mutations in thymoma and thymic carcinoma have not been widely reported. Until now, only one study by Wheler et al reported a *PIK3CA* mutation in one of the 12 TET patients.²⁴ In the present cohort, two *PIK3CA* mutations were detected in thymoma with a frequency of 3.8%. Epidermal growth factor receptor (*EGFR*) mutations have been identified as driver genes in non-small-cell lung cancer (NSCLC) at a frequency of 40%–50% in the East Asian population. The *EGFR* mutations in TET have been identified in previous studies.^{25,26} No representative clinical characteristics associated with *EGFR* mutations were found, although most patients were diagnosed with thymoma but not thymic carcinoma. In the current study, our patient who carried the *EGFR* mutation represented type B3.

No targeted drugs are approved in TET due to its rarity and lack of explicit molecular targets. Patients with advanced/metastatic thymoma or thymic carcinoma benefited from mammalian target of rapamycin inhibitor-based therapy in a Phase I study with a median time to treatment failure of 11.6 months.²⁴ However, no *PIK3CA* mutation was found in patients responding to *mTOR* inhibitor treatment. Higher serum concentrations of *VEGF*, *b-FGF*, and *cKIT* expression were observed in patients with thymic carcinoma. One Phase II trial investigated the activity of sunitinib, an

oral tyrosine kinase inhibitor, including *VEGFR*, *KIT*, and *PDGFR* in TET patients.²⁷ The results showed that sunitinib showed clinical activity in heavily pretreated patients with thymic carcinoma. EGFR-tyrosine kinase inhibitors (TKIs) displayed adequate efficacy in NSCLC patients who carried *EGFR* mutations. A Phase II study of gefitinib for advanced thymic malignancies showed that disease was controlled in 15 patients, including partial response in one case and stable disease in 14 others.²⁵ However, no association was found between *EGFR* mutations and gefitinib efficacy. The role of *EGFR* mutations in carcinomas may be different in thymoma compared with NSCLC. In our cohort, only one patient with thymoma carried the *EGFR* mutation. Unfortunately, no EGFR-TKI was administered to this patient, and the efficacy of EGFR-TKI is unknown.

The prognostic role of clinical and pathologic factors remains controversial in TET.^{2-4,16-17} The Masaoka pathological stage represents an important prognostic factor for thymic carcinoma. In the current study, the Masaoka pathological stage was associated with significant effects on the prognosis of thymoma and thymic carcinoma. In addition, we found that histological subtypes hold the key to survival in patients with TET. However, other clinical factors, such as sex, age, and myasthenia gravis, played no role in survival.

The occurrence of extrathymic malignancy in TET patients has not been delineated in the published studies, with an incidence of ~10% having been reported.^{28,29} Four of 52 patients were found with extrathymic malignancy in the present study, which is consistent with previous studies.

Limitations

The major limitation of this study was the relatively small sample size to evaluate gene status and survival. Therefore, a larger series of patients and high-throughput sequencing with additional genes are needed to identify the prevalence of mutations and survival in TET. Furthermore, functional analyses of the mutations are needed to determine the molecular mechanisms of tumorigenesis.

Conclusion

In conclusion, this study reports the incidence of *PIK3CA* and *EGFR* mutations in some TET patients. These mutations may represent promising therapeutic candidates. In addition to Masaoka stage, histological subtypes predict the survival of patients with TET.

Disclosure

The authors report no conflicts of interest in this work.

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