BRIEF REPORT

Trophoblast Cell Surface Antigen 2 Expression in Thymic Carcinoma: Brief Report

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ABSTRACT

Introduction: Trophoblast cell surface antigen 2 (TROP2) is a transmembrane glycoprotein overexpressed in various cancer types. Although TROP2-targeting therapy is currently attracting attention, little is known about TROP2 expression in thymic carcinoma.

Methods: TROP2 gene expression in thymic epithelial tumors was analyzed using RNA-sequencing (RNA-seq) data for 122 cases obtained from The Cancer Genome Atlas. Immunohistochemistry (IHC) staining with anti-TROP2 antibody (SP295) was performed in 26 cases of thymic carcinoma tissues surgically resected at Juntendo University.

Results: RNA-seq data analysis from The Cancer Genome Atlas revealed that *TACSTD2* (gene encoding TROP2) expression was significantly higher in thymic carcinoma than in thymoma (adjusted $p = 6.64e\text{-}05$). There was also a trend of increasing expression in the order of thymoma type B1, B2, B3, and thymic carcinoma. As for IHC in thymic carcinoma, TROP2 expression was localized to the membrane of cancer cells. Intensity 0, 1, and 2 was observed in six (23.1%), 11 (42.3%), and nine (34.6%) cases, respectively, leading to TROP2 positivity in 20 cases (76.9%). The median proportion of TROP2-positive tumor cells and the median H-score were 25.0% (range: 0%–100%) and 25.0 (range: 0–200), respectively. No relevant factors were identified in the analysis of TROP2 expression and patient background. Although not significant, high TROP2 expression (H-score $\geq$ 50) tended to be associated with shorter survival.

Conclusions: TROP2 expression in thymic carcinoma was confirmed by both RNA-seq and IHC, with high expression observed in IHC for intensity (76.9%) and proportion. TROP2 could be a potential target in thymic carcinoma.

Keywords: TROP2; Thymic carcinoma; Immunohistochemistry; RNA-seq

Introduction

Thymic epithelial tumor is a rare disease, arising in the anterior mediastinum and originating from thymic

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epithelium.[1] Especially in thymic carcinoma, advanced or recurrent cases have limited prognosis. Cytotoxic chemotherapy, multitargeted tyrosine kinase inhibitors, and immune checkpoint inhibitors have limited efficacy, with an objective response rate of 20% to 40%.[2] Therefore, targets for treating thymic carcinoma warrant exploration. Trophoblast cell surface antigen 2 (TROP2) is a 35-kDa type 1 transmembrane protein and is overexpressed in several cancers.[3,4] Recently, TROP2 has gained attention because antigen-drug conjugates targeting TROP2 have been developing.[5–9] Nevertheless, TROP2 expression in thymic carcinomas remains unclear. In this study, we evaluated TROP2 expression and its association with clinicopathologic findings in thymic carcinoma.

Materials and Methods

The Cancer Genome Atlas Data Analysis

RNA-sequencing (RNA-seq) data and clinical information on thymic carcinoma, thymoma, and normal thymus were derived from The Cancer Genome Atlas (TCGA) database (TCGA-THYM) up to August 2023. Differential expression analysis of TROP2 was performed with DESeq2. The enrollment conditions for the differentially expressed gene were $\log_2$ fold change greater than 1 and adjusted p values less than 0.05.

Immunohistochemistry

Paraffin-embedded tissue samples were obtained from patients with thymic carcinoma who underwent surgery at Juntendo University Hospital, Tokyo, Japan, from May 1986 to November 2017. Pathologic diagnoses of thymic carcinoma were based on the 2021 WHO classification of thoracic tumors.[10] TROP2 was stained using anti-TROP2 rabbit monoclonal antibody (clone: SP295, ab227691, Abcam, Cambridge, United Kingdom) and Benchmark XT automated stainer (Roche, Basel, Switzerland). After section baking and deparaffinization, antigens were retrieved using a Tris-based buffer in a microwave oven at 100°C for 56 minutes. Anti-TROP2 antibody was applied to the section and incubated for 60 minutes. Sections were counterstained with hematoxylin. Tissues from the tonsils were used as positive and negative controls. For the evaluation of TROP2 expression, the intensity, proportion of positive cells, and H-score in the tumor were used. The intensity of TROP2 expression was defined as 0 (absent), 1 (weak to moderate), or 2 (strong), as previously reported.[8] Intensity was evaluated when the proportion of positive tumor cells was 10% or greater. The H-score was defined as the product of intensity and the proportion of positive cells. Two pathologists (TH and SK), blinded to the clinical data, independently reviewed all stained sections. When discrepancies arose, the slides were reviewed using a multiheaded microscope to reach a consensus. The use of the patient samples and clinical data in this study was approved by the institutional review board of Juntendo University Hospital (20-082) and was conducted in accordance with the Declaration of Helsinki and its later amendments. The requirement for consent to participate was waived owing to the retrospective nature.

Clinical Data

We retrospectively reviewed patients' clinical information from medical records, including sex, age at the time of surgery, smoking history, histologic diagnoses, Masaoka stage, resection status, recurrence status, date of disease progression and death, and last contact if death did not occur at the cutoff date (June 30, 2023). Overall survival (OS) was defined as the period from the date of surgery to death or last follow-up.

Statistical Analysis

Fisher's exact test was used to evaluate categorical values. Spearman's rank correlation coefficient was calculated to assess the association between variables. The Kaplan-Meier method, log-rank test, and univariate Cox regression analysis were used for survival analysis. Statistical data were analyzed using R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and jamovi version 2.3.1 (https://www.jamovi.org/). Statistical significance was set at p values less than 0.05.

Results

TACSTD2 (TROP2) Gene Expression in the TCGA-THYM Cohort

We collected RNA-seq and clinical information on thymic epithelial tumors from TCGA-THYM, including 109 thymoma, 11 thymic carcinoma, and two normal thymus samples. The expression of *TACSTD2* (gene encoding TROP2) in the TCGA-THYM cohort was significantly higher in thymic carcinoma than in thymoma (adjusted $p = 6.064\text{e-}05$; Fig. 1). Moreover, there was a tendency for expression to increase in the order of thymoma type B1, B2, B3, and thymic carcinoma (Supplementary Fig. 1). Survival analysis was not conducted owing to few survival events. Because of the higher gene expression of TROP2 in thymic carcinoma, we subsequently focused our analysis on thymic carcinoma.

TROP2 Expression on Immunohistochemistry

Owing to insufficient knowledge of the optimal evaluation method, we assessed the intensity, proportion of positive tumor cells, and H-score of TROP2 in thymic carcinoma. TROP2 expression was observed mainly in

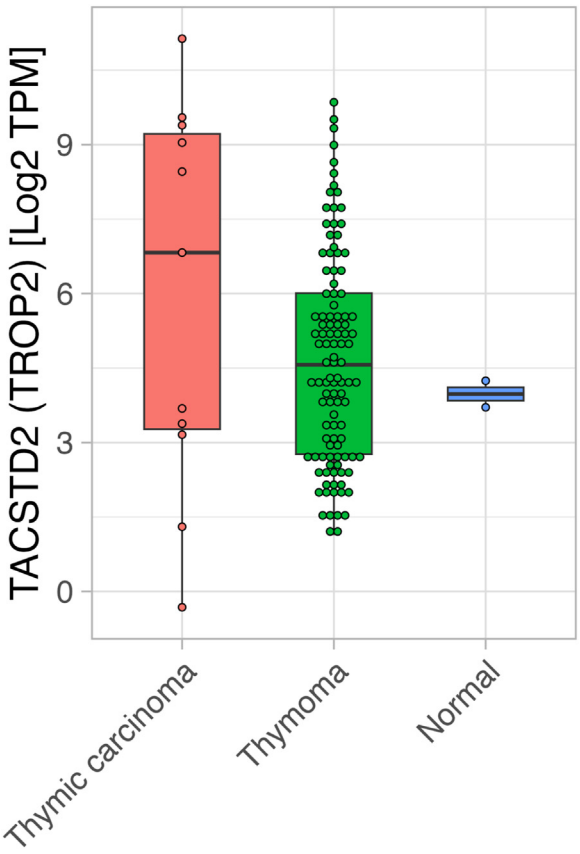

Figure 1. *TACSTD2* (TROP2) gene expression in thymic carcinoma, thymoma, and normal thymus in The Cancer Genome Atlas data set. TPM, transcripts per million; TROP2, trophoblast cell surface antigen 2.

the cell membrane of cancer cells. The proportions of intensity 0, 1, and 2 were six (23.1%), 11 (42.3%), and nine (34.6%), respectively (Fig. 2). When TROP2 positivity was defined as intensity 1 and 2, 20 patients (76.9%) had positive results for TROP2, and six patients (23.1%) had negative results (intensity 0). The proportion of positive tumor cells was a median of 25.0% (range: 0%–100%), and TROP2 expression in more than 10% of tumor cells was observed in 20 cases (76.9%). There was a high correlation between intensity and tumor cell proportion ($p \leq 0.0001$, Spearman's rho = 0.8363) (Supplementary Fig. 2). The H-score had a median of 25.0 (range: 0–200). When using an H-score of 50 or higher as the cutoff, high TROP2 expression was observed in eight cases (30.8%).

TROP2 Expression and Clinical Data

Next, we investigated the relationship between TROP2 expression and patient background and survival outcome. The patient background is presented in Table 1, with 14 (53.8%) being male and the median age 63 years (range: 36–79). Fifteen (60.0%) had stage IV disease. The median follow-up period for all censored cases was 109.0 months (range: 0.2–227.3). We evaluated the relationship among patient background and intensity (cutoff 1/2 versus 0), the proportion of positive tumor cells (≥25% versus <25%), and H-score (≥50 versus <50), but no significant factors were found (Supplementary Table 1). Nevertheless, although not statistically significant, there was a trend toward shorter OS in the group with H-score of 50 or greater than in the group with H-score lower than 50 (median OS, 88.0 versus 208.0 mo, $p = 0.333$) (Supplementary Fig. 3).

Discussion

In this study, we analyzed RNA-seq data for 122 thymic epithelial tumors from TCGA and found higher TROP2 gene expression in thymic carcinoma than in thymoma. In immunohistochemistry (IHC) analysis of 26 thymic carcinomas, 76.9% had an intensity of 1 or higher. There was a strong correlation between intensity and proportion of positive tumor cells.

TROP2 has been reported to be involved in tumor progression and metastasis.[3,4] The association between TROP2 and the prognosis of cancer has been evaluated in multiple cancer types, and most reports indicate high TROP2 expression correlates with poor prognosis.[4] Our study using TCGA data revealed that TROP2 gene expression was higher in thymic carcinoma than in thymoma, and that gene expression tended to increase in the order of B1 to B3 and thymic carcinoma. Although there was no significant difference, OS tended to be shorter in patients with higher H-scores, suggesting an involvement of TROP2 in the progression of thymic carcinoma.

Little was known about TROP2 expression in the thymus and thymic epithelial tumors until recently, except for a report that TROP2 expression was not observed in the normal thymus except in medullary epithelial cells (Hassall's corpuscles).[11] In the present study, IHC evaluation of 26 thymic carcinomas revealed that TROP2 was expressed on tumor cells with an intensity of 1 or higher in 76.9% of cases. In a recently reported study of 30 thymic epithelial tumors, 13 thymic carcinomas were evaluated, and all of them expressed TROP2 intensity 1 or higher (1+: three carcinomas [23%], 2+: seven carcinomas [54%], 3+: three carcinomas [23%]).[12] In that study, the percentage of patients with intensity 1 or higher was higher than in our study, possibly because the number of cases was limited. Our study included 26 thymic carcinoma cases; we further evaluated the proportion of positive tumor cells and H-score and observed TROP2 expression of more than 10% in tumor cells in 20 cases. Meanwhile, the appropriate cutoffs for intensity and proportion, H-score, and the best method of assessment remain unclear. In this study,

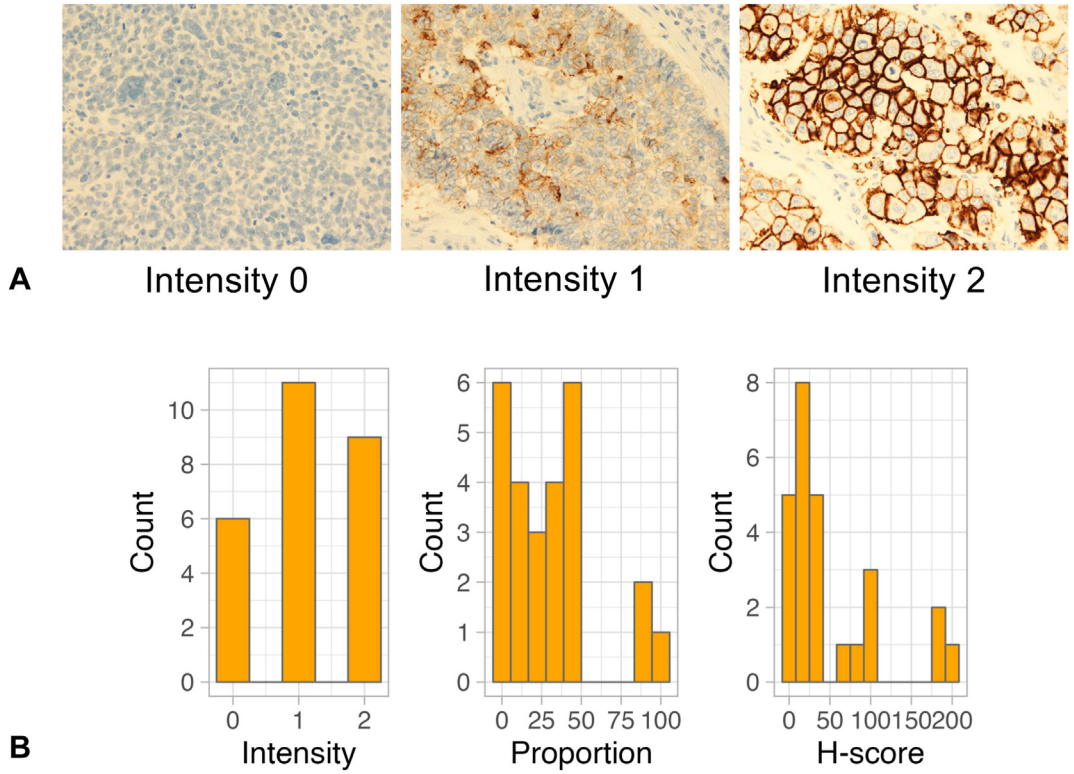

Figure 2. Immunohistochemical evaluation of TROP2 expression. *(A)* Representative images of each intensity of TROP2. Intensity 0: absent; Intensity 1: weak to moderate; Intensity 2: strong. *(B)* Distributions of intensity, proportion, and H-score of TROP2 expression. TROP2, trophoblast cell surface antigen 2.

we evaluated both parameters and found no statistically significant correlation, except that OS tended to be shorter in patients with a higher H-score. Furthermore, the significance of TROP2 expression as a biomarker of the efficacy of antigen-drug conjugates is inconclusive. One report suggested a possible correlation between sacituzumab govitecan efficacy and TROP-2 H-score in triple-negative breast cancer, whereas another reported no association with dapotomab deruxtecan efficacy in NSCLC.[9,13] Further studies are required to establish a classification for staining.

Our study has some limitations, including being a single-institution retrospective study with a small sample size that included older samples. Nevertheless, thymic carcinoma is a rare disease, and even with large-scale databases, the sample size remains small. Therefore, despite its small sample size, the study may offer important evidence. In addition, TROP2 expression was observed even in the oldest samples, making them valuable for evaluation.

In conclusion, TROP2 expression in thymic carcinoma was confirmed by both RNA-seq and IHC, with high expression observed in IHC for intensity and proportion. TROP2 could be a potential target for thymic carcinoma.

Table 1. Baseline Characteristics

Characteristics	Overall (N = 26)
Age, y, n (%)	
<70	20 (76.9)
≥70	6 (23.1)
Sex, n (%)	
Male	14 (53.8)
Female	12 (46.2)
Smoking status, n (%)	
Current or former	13 (50.0)
Never or not known	13 (50.0)
Histologic diagnosis, n (%)	
Squamous cell carcinoma	26 (100.0)
Masaoka stage, n (%)	
I-III	14 (40.0)
IV	12 (60.0)

CRediT Authorship Contribution Statement

Kana Kurokawa: Conceptualization, Formal analysis, Investigation, Data Curation, Writing - original Draft, visualization.

Tetsuhiko Asao: Conceptualization, Methods, Validation, Investigation, Writing - review & editing, Project administration.

Takuo Hayashi: Methods, Validation, Investigation, Writing - review & editing.

Satsuki Kishikawa: Methods, Validation, Investigation, Writing - review & editing.

Koichiro Kanamori: Investigation, Resources, Data curation, Writing - review & editing.

Yosuke Miyashita: Investigation, Resources, Data curation, Writing - review & editing.

Ikuko Nakamura: Investigation, Resources, Data curation, Writing - review & editing.

Takehito Shukuya: Data curation, Writing - review & editing.

Taichi Miyawaki: Data curation, Writing - review & editing.

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Yoichiro Mitsuishi: Data curation, Writing - review & editing.

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Naoko Shimada: Data curation, Writing - review & editing.

Fumiyuki Takahashi: Data curation, Writing - review & editing.

Kazuya Takamochi: Resources, Writing - review & editing.

Kenji Suzuki: Resources, Writing - review & editing, Supervision.

Kazuhisa Takahashi: Writing - review & editing, Supervision.

Disclosure

Dr. Asao reports honoraria from AstraZeneca K.K., Bristol-Myers K.K., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Eli Lilly Japan K.K, Merck Biopharma Co., Ltd., Merck Sharp & Dohme K.K, Nippon Boehringer Ingelheim Co., Ltd., Nippon Kayaku Co., Ltd., Ono Pharmaceutical Co., Ltd., Pfizer Inc., Taiho Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Company Limited outside of the submitted work. Dr. Shukuya reports grants and honoraria from AstraZeneca K.K., Chugai Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K.K., and Merck Sharp & Dohme K.K. and honoraria from Taiho Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Ono Pharmaceutical Co., Ltd., Bristol-Myers Squibb Company, Nippon Kayaku Co., Ltd., Takeda Pharmaceutical Company, Pfizer Inc., and Eisai Co., Ltd. outside of the submitted work. Dr. Fumiyuki Takahashi reports grants from AstraZeneca, Nippon Boehringer Ingelheim, Merck Sharp & Dohme, Novartis, and Lilly Japan outside of the submitted work. Dr. Kazuhisa Takahashi reports grants and honoraria from Chugai Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Pfizer Inc., Taiho Pharmaceutical Co., Ltd., Kyorin Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Novartis Pharma K.K., Eli Lilly Japan K.K., Nippon Kayaku Co., Ltd., Takeda Pharmaceutical Company Limited, and grants from Nippon Shinyaku Co., Ltd., Tsumura & Co., Teijin Pharma Limited, Sanofi K.K., Shionogi & Co., Ltd., Bayer Yakuhin, Ltd., Daiichi Sankyo Co., Ltd., Nipro Pharma Corporation, Asahi Kasei Pharma Corporation, Kyowa Kirin Co., Ltd., and honoraria from Merck Sharp & Dohme K.K., AstraZeneca K.K., Merck Biopharma Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Bristol-Myers K.K., Meiji Seika Pharma Co., Ltd., Viatris Inc., Janssen Pharmaceutical K.K., Abbott Japan LLC, and Thermo Fisher Scientific Inc. outside the submitted work. The remaining authors declare no conflict of interest.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at [https://doi.org/10.1016/j.jtocrr.2024.100693].

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