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Original Article

Overexpression of trophoblast cell surface antigen 2 is associated with BRAF V600E mutation and aggressive behavior in papillary thyroid cancer

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Abstract: Background: Papillary thyroid cancer (PTC) is the most common endocrine tumor with an increasing incidence. The dilemma in treating this cancer is how to discriminate the tumors with aggressive behavior from the indolent ones. Trophoblast cell surface antigen 2 (trop-2) has been identified in multiple epithelial cancers and proven to be associated with shortened overall survival and disease-free survival. Methods: In this study, we retrospectively collected the clinicopathologic characteristics of 145 patients who underwent surgery at Fudan University Shanghai Cancer Center (FUSCC), and a validation cohort from The Cancer Genome Atlas (TCGA) database was identified to support the conclusion. Quantitative real-time polymerase chain reaction (qPCR) was employed to determine trop-2 mRNA in cancer tissue and adjacent normal tissue of PTC. BRAFV600E mutation analysis was determined using Sanger sequencing. The correlation of trop-2 and clinical characteristics was analyzed with Mann-Whitney U, Kruskal-Wallis, χ^2, and Fisher's exact tests. Results: Trop-2 overexpression was significantly associated with lymph node metastasis (LNM) (FUSCC, P<0.001; TCGA, P<0.001) and extrathyroidal extension(ETE) (FUSCC, P=0.006; TCGA, P<0.001) in both the FUSCC and TCGA cohorts. It also proved to be an independent risk factor for LNM and ETE in multivariate analysis in both cohorts. Furthermore, trop-2 expression was also associated with BRAFV600E mutation, P<0.001 in both cohorts. Conclusions: The study found that trop-2 overexpression was correlated with LNM and ETE. Additionally, it was associated with BRAF mutation, therefore, trop-2 overexpression was a potential biomarker for aggressive behaviors of PTC, also it could be an indication for targeted therapy.

Keywords: Papillary thyroid carcinoma, trop-2, lymph node metastasis, extrathyroidal extension

Introduction

Thyroid cancer is the most common malignancy in the endocrine system. The incidence has increased rapidly in the past few decades [1]. It has four major pathology types including: papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), medullary thyroid cancer (MTC), and anaplastic thyroid cancer (ATC). PTC is the most frequently diagnosed type of thyroid cancer and contributes most to its increasing incidence [1]. It is usually slow growing, indolent and rarely fatal, most cases are curable with surgery and radioactive iodine therapy (RAI), but, there are also some patients suffering from local advanced, metastatic, or radioiodine resistant tumor that lack effective treatment. The dilemma is how to discriminate the aggressiveness of each individual case, in order to personalize initial therapy and surveillance. The BRAFV600E mutation is the most common genetic alteration in PTC and is considered as a biomarker of adverse clinical features at the beginning. Unfortunately, recent studies did not support it as an independent risk factor [2], therefore, new biomarkers that can predict the aggressiveness of PTC are still needed.

The overexpression of trophoblast cell surface antigen 2 (trop2) has been reported in PTC [3, 4], which is a transmembrane glycoprotein (also referred to as M1S1, TACSTD2, EGP-1), coded by the gene Tacstd2 [5, 6]. It was first discovered in trophoblast cells that possess the ability to invade uterine decidua during placental implantation [7]. In many types of cancer such as non-small cell lung cancer [8], colon cancer

[9], and prostate cancer [10], its overexpression is associated with poor prognosis, but similar correlation has not been shown in pancreatic cancers [11]. Trop-2 has been implicated in numerous intracellular signaling pathways including mitogen activated protein kinase (MAPK) signaling pathway, which is a critical characteristic of PTC [12], but the functional role of trop-2 in PTC has not been fully elucidated. To determine whether trop-2 could be a promising biomarker for prognosis and a candidate for the target of immunotherapy in PTC, we enrolled many patients both from FUSCC and patients from TCGA database in this study, in whom the association between Trop-2 and the clinicopathological characteristic of PTC has been thoroughly studied.

Materials and methods

Patients and clinicopathological data

145 paired samples were randomly selected from the tissue bank at Fudan University Shanghai Cancer Center (FUSCC), the specimens were collected from those patients who were treated for thyroid cancer in FUSCC between January 2012 to December 2013. Patients' clinical characteristics including sex, age at diagnosis, maximum diameter of tumor, prime tumor laterality, extrathyroidal extension (ETE), and cervical lymph node metastasis (LNM) were retrospectively collected from patient records. All the patients were staged using the 2009 TNM classification of the American Joint Committee on Cancer/International Union Against Cancer [13]. The histological characteristics were confirmed by two pathologists independently. Each patient provided a written informed consent for his/her specimens and information to be used for research and stored in the hospital database; this study was approved by the Ethical Committee of FUSCC. All the procedures performed in our study were in accordance with the ethical standards of our institutional research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. In addition, a validation cohort from The Cancer Genome Atlas (TCGA) database was identified to confirm the preliminary findings at FUSCC. A total of 382 primary PTC patients with detailed trop-2 expression, BRAFV600E mutation, and clinical data were collected from the updated TCGA database. The TCGA cohort data were available on the website of cBioportal (https://cbioportal.org), and the gene expression dataset and clinical data were obtained from the file named TCGA_THCA_exp_HiSeqV2_PANCAN. Those patients without complete records were excluded.

Tissue array and immunohistochemical staining

Tissue arrays were purchased from Biomax, TH241 and TH242 were used to test the antibodies and conditions. The staining results of TH80 and TH241 were useed for analysis. Immunohistochemical staining was carried out according to appropriate protocols. First, tissue sections were deparaffinized in xylene and hydrated through descending concentrations of ethanol before being placed in blocking solution to inhibit endogenous peroxidase activity. The slides were then incubated with primary antibody (HPA055067 1:300; Sigma-Aldrich, Inc.) at 4°C overnight. A horseradish peroxidase-conjugated rabbit secondary antibody was added for 60 min at room temperature, followed by 3,3'-diaminobenzidine (DAB) development (DAB Substrate Chromogen System, Dako) and hematoxylin and eosin (H&E) as per standard staining protocol. Slides were fixed and images obtained with the Olympus IX71 inverted microscope using the DP2-BSW Olympus image acquisition software system. The results were confirmed by two experienced pathologists who were blinded to the clinicopathologic data of the patients.

RNA extraction and RT-PCR

Total RNA was extracted from tissues with TRIzol Reagent (Invitrogen, Inc.). The quality and integrity of RNA was evaluated by A260/A280 ratio and 18 s/28 s band by agarose electrophoresis. 1 μg of total RNA was used for first-strand DNA synthesis employing a PrimeScriptTM RT reagent kit (Takara Bio, Inc., Japan). Real-time PCR was performed in triplicate by according to the SYBR Green PCR method, with a SYBR Premix Ex TaqTM kit (Takara Bio, Inc., Japan) and in accordance with the manufacturer's instructions. The primers for the interested genes (TACSTD2) were synthesized by Sangon Company (Sangon Biotech Co., Ltd., Shanghai, China), the primers were as follows: (forward: 5'-TATTACCTGGACGAGATTCCCC-3', reverse: 5'-CCGACTTTCTCCGGTTGGT-3'). The actin was used as internal control for mes-

Table 1. Correlation between Trop-2 expression and clinicopathologic characteristics in PTC in the FUSCC and TCGA cohorts

Variable	FUSCC cohort (N=145)				TCGA cohort (N=382)			
	N	Low	High	*P*-value	N	Low	High	*P*-value
Sex				0.407				0.319
Male	54	26 (48.1%)	28 (51.9%)		97	51 (52.6%)	46 (47.4%)	
Female	91	47 (51.6%)	44 (48.4%)		285	140 (49.1%)	145 (50.9%)	
Age (years)				0.342				0.500
<55	88	46 (52.3%)	42 (47.7%)		257	128 (49.8%)	129 (50.2%)	
≥55	57	27 (47.4%)	30 (52.6%)		125	63 (50.4%)	62 (49.6%)	
Maximum size of tumor (cm)				0.035				0.011
<2	96	52 (54.2%)	44 (45.8%)		111	60 (54.1%)	51 (45.9%)	-
2-4	43	21 (48.8%)	22 (51.2%)		129	74 (57.4%)	55 (42.6%)	
>4	6	0 (0.0%)	6 (100.0%)		142	57 (40.1%)	85 (59.9%)	
Primary tumor laterality				0.381				0.066
Unilateral	100	49 (49.0%)	51 (51.0%)		318	165 (51.9%)	153 (48.1%)	
Bilateral	45	24 (53.3%)	21 (46.7%)		64	26 (40.6%)	38 (59.9%)	
Histological type				0.748				<0.001
Classical PTC	143	72 (50.3%)	71 (49.7%)		268	112 (41.8%)	156 (58.2%)	
Follicular PTC	2	1 (50%)	1 (50%)		83	71 (85.5%)	12 (14.5%)	
Tall-cell PTC	-	-	-		29	7 (24.1%)	22 (75.9%)	
Other types	-	-	-		2	1 (50%)	1 (50%)	
ETE				0.006				<0.001
Yes	38	12 (31.6%)	26 (68.4%)		116	38 (32.8%)	78 (67.2%)	
No	107	61 (57.0%)	46 (43.0%)		266	153 (57.5%)	113 (42.5%)	
LNM				<0.001				<0.001
N0	51	36 (70.6%)	15 (29.4%)		205	122 (59.5%)	83 (40.5%)	
N1	94	37 (39.4%)	57 (60.6%)		177	69 (39.0%)	108 (61.0%)	
T stage				0.233				0.002
T1–T2	110	53 (48.2%)	57 (51.8%)		240	134 (55.8%)	106 (44.2%)	
T3–T4	35	20 (57.1%)	15 (42.9%)		142	57 (40.1%)	85 (59.9%)	
TNM stage				0.669				0.001
I	105	56 (53.3%)	49 (46.7%)		220	120 (54.5%)	100 (45.5%)	
II	2	1 (50.0%)	1 (50.0%)		42	28 (66.7%)	14 (33.3%)	
III	20	9 (45.0%)	11 (55.0%)		80	30 (37.5%)	50 (62.5%)	
IV	18	7 (38.9%)	11 (61.1%)		40	13 (32.5%)	27 (67.5%)	
BRAFV600E				<0.001				<0.001
Mutation	63	21 (33.3%)	42 (66.7%)		227	49 (21.6%)	178 (78.4%)	
Wild-type	36	19 (52.8%)	17 (47.2%)		155	142 (91.6%)	13 (8.4%)	

Note: ETE, extrathyroidal extension; LNM, lymph node metastasis.

senger RNA (mRNA) assays. The threshold cycle (Ct) values were analyzed using the comparative Ct (-ΔCt) method. The level of targets was obtained by normalizing to the endogenous reference and relative to a control.

DNA and BRAFV600E mutation analysis

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. The DNA template was amplified for analysis of mutations in exon 15 of the BRAF gene using PCR protocol as previously mentioned, the primers were as follows: (forward: 5'-TCATAATGCTTGCTCTGATAGGA-3', reverse: 5'-GGCCAAAAATTTAATCAGTGGA-3'), followed by a Big Dye (Thermo Fisher Scientific) reaction for Sanger sequencing. BRAFV600E mutation was recognized on sequencing electropherograms.

Statistical analysis

Categorical data were summarized with frequencies and percentages. The continuous results were expressed as mean ± standard devi-

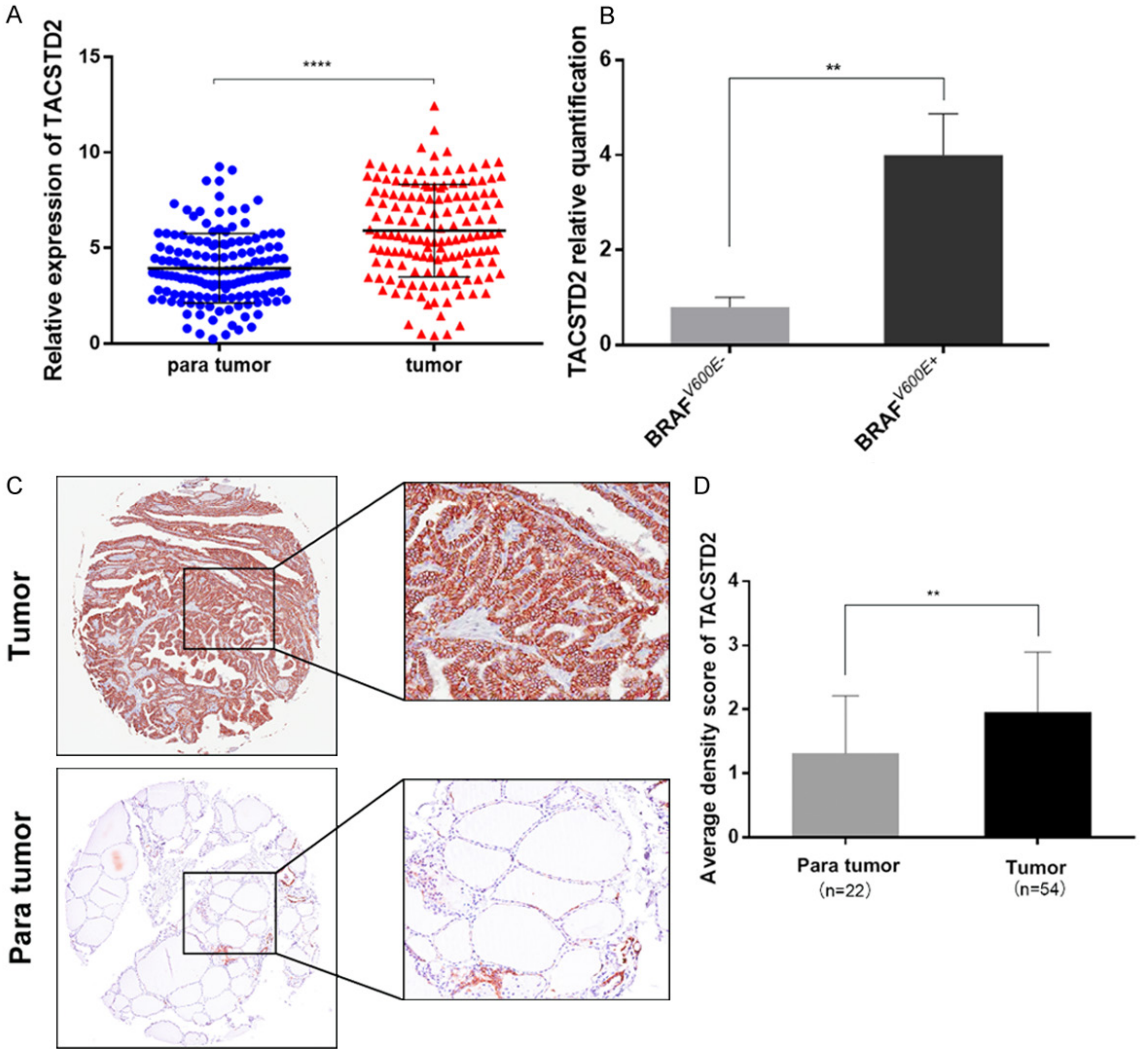

Figure 1. Expression of trop-2 in PTC and normal thyroid tissue. A. Trop-2 mRNA expression was significantly elevated in PTC compared with the level in the paired adjacent normal tissues (P<0.001) from FUSCC cohort. Trop-2 mRNA expression was normalized for the actin mRNA level (2-ΔCt). The relative quantification was measured as log10 values. B. Trop-2 mRNA expression was significantly elevated in PTC with BRAFV600E mutation compared BRAF wild type (P<0.001). C, D. Immunohistochemistry analysis of tissue array containing PTC samples and paired normal tissue samples. Trop-2 protein was extensively expressed in PTC while normal thyroid epithelial cells rarely had a positive stain (P=0.022).

ation (SD). Paired and independent Student's t-tests were used to compare continuous variables in two groups. Associations between continuous variables and categorical variables were evaluated using Mann-Whitney U-test for two groups and Kruskal-Wallis test for more than two groups. χ^2 and Fisher's exact test were used for categorical variables. To verify the associations between trop-2 and BRAFV600E mutation and other characteristics, patients were divided into two subgroups (low expression and high expression) according to the median value of Trop-2 expression at the mRNA level in each cohort. A nonparametric receiver operating characteristic (ROC) analysis was performed to calculate the best cutoff value for trop-2 expression level that would be predictive of LNM and ETE. Moreover, univariate and multivariate analyses were performed to determine the risk factors for LNM in PTC in the FUSCC and TCGA cohorts using a logistic regression calculated by odds ratio (OR) and 95% confidence interval (CI). The area under a receiver characteristic curve was used to measure the relative predictability of independent factors for LNM. A *P*-value less than 0.05 was

Table 2. Clinicopathologic and molecular factors associated with LNM in PTC in the FUSCC cohort

Variables	Univariate analysis			Multivariate analysis		
	P-value	OR	95% CI for OR	*P*-value	OR	95% CI for OR
Female	0.283	0.674	0.328-1.385			
Age ≥55 years	0.487	0.782	0.391-1.566			
Tumor size (cm)						
2-4	0.254	3.571	0.402-31.753			
>4	0.039	2.357	1.043-5.328	0.064	2.359	0.951-5.849
Multifocality	0.660	1.179	0.567-2.448			
ETE	0.351	0.682	0.306-1.523			
BRAFV600E	0.044	2.500	1.027-6.087	0.191	1.844	0.737-4.614
Trop-2 (greater than cutoff)*	<0.001	4.122	1.944-8.741	0.005	3.201	1.428-7.176

Note: ETE, extrathyroidal extension. *cutoff = 0.261.

considered significant. Statistical analyses were performed using the SPSS for Windows (SPSS Inc., Chicago, IL, USA).

Results

Clinicopathological data of patients in the FUSCC and TCGA cohorts

A total of 145 patients were enrolled from FUSCC, the clinicopathological data was showed in **Table 1**, there were 54 males and 91 females, the median age was 41.7±12 ranged from 14 to 72 years. The maximum diameter of the primary tumor was 1.738±0.901 cm on average. 94 of 145 (64.82%) patients had LNM and 38 of 145 (26.2%) patients had ETE. 382 PTC patients were collected from the TCGA database as a validation cohort, there were 97 males and 285 females, the median age was 47.21±15.6 range from 17 to 89 years, LNM was present in 177 of 382 (46.34%) patients, and ETE occurred in 116 of 382 (30.37%) patients. The ratios of subtypes of PTC like classic, follicular, and tall-cell were 71.15%, 21.73% and 7.59%, respectively.

Expression of trop-2 is upregulated in PTC in vivo

Trop-2 mRNA expression was detected using RT-PCR in the total RNA extracted from cancer tissues and paired normal tissues in FUSCC cohort, as show in **Figure 1A**, trop-2 mRNA expression was significantly elevated in PTC compared to paired normal thyroid tissue (P<0.001). Trop-2 protein level was detected using immunohistochemistry on the tissue array containing PTC samples and paired normal tissue samples. As shown in **Figure 1C** and **1D**, trop-2 protein was extensively expressed in PTC, while the normal thyroid epithelial cells rarely were positive (P=0.022).

Overexpression of trop-2 is correlated with LNM in PTC

As shown in **Table 1**. Trop-2 expression level was median separated into low and high groups, in both the FUSCC and TCGA cohorts, higher level of trop-2 expression was associated with LNM, ETE, maximum diameter and BRAFV600E mutation status. There was also a significant difference in trop-2 expression among different TNM stages (P=0.001), histologic types (P<0.001), and (P<0.001) in the TCGA cohort. Sex andage, failed to correlate with trop-2 in either cohort.

Further analysis aimed to determine whether overexpression of trop-2 was an independent risk factor for LNM in PTC was conducted. ROC analysis identified the best cutoff of trop-2 expression levels that were predictive of LNM was 0.261 in FUSCC cohort, area under ROC curve is 0.669. **Table 2** shows in FUSCC cohort, tumor size more than 4 cm (OR=2.357, 95% CI 1.043-5.328, P=0.039), BRAFV600E mutation (OR=2.5, 95% CI 1.027-6.087, P=0.044) and trop-2 expression greater than 0.261 (OR= 4.122, 95% CI 1.944-8.741, P<0.001) was a risk factor for LNM in univariate analysis. After adjusting for tumor size and BRAF600E mutation, trop-2 expression greater than the cutoff value (OR=3.201, 95% CI 1.428-7.176, P= 0.005) was found to be an independent risk factor for LNM, but tumor size and BRAF600E was not significant by multivariate analysis.

Table 3. Clinicopathologic and molecular factors associated with LNM in PTC in the TCGA cohort

Variable	Univariate analysis			Multivariate analysis		
	P-value	OR	95% CI for OR	*P*-value	OR	95% CI for OR
Female	0.058	0.639	0.402-1.016	0.166	0.694	0.414-1.164
Age ≥55 years	0.196	0.752	0.489-1.158			
T stage (T3-4)	<0.001	3.044	1.978-4.684	0.040	2.068	1.032-4.142
Histological type						
Follicular PTC	<0.001	0.304	0.174-0.530	0.061	0.531	0.274-1.029
Tall-cell PTC	0.265	1.565	0.712-3.439	0.788	1.125	0.478-2.650
ETE	<0.001	3.277	2.073-5.182	0.272	1.517	0.721-3.192
BRAFV600E	<0.001	2.427	1.589-3.708	0.055	2.090	0.983-4.444
Trop-2 (greater than cutoff)*	<0.001	4.018	2.536-6.366	<0.001	5.126	2.344-11.210

Note: ETE, extrathyroidal extension. *cutoff = -0.655.

Table 4. Clinicopathologic and molecular factors associated with ETE in PTC in the FUSCC cohort

Variables	Univariate analysis			Multivariate analysis		
	P-value	OR	95% CI for OR	*P*-value	OR	95% CI for OR
Female	0.953	1.023	0.475-2.203			
Age ≥55 years	0.258	1.573	0.718-3.445			
Tumor size (cm)						
2-4	0.196	3.000	0.567-15.867	0.413	2.110	0.353-12.621
>4	0.942	1.031	0.451-2.356	0.969	0.982	0.392-2.462
Multifocality	0.623	0.821	0.373-1.804			
LNM	0.351	0.682	0.306-1.523	0.895	1.063	0.431-2.621
BRAFV600E	0.314	1.628	0.631-4.201	0.726	0.810	0.250-2.628
Trop-2 (greater than cutoff)*	<0.001	4.611	2.103-10.108	<0.001	4.605	1.923-11.028

Note: LNM, lymph node metastasis. *cutoff = 0.479.

Table 3 shows that in TCGA cohort, the best cutoff was determined to be -0.655, area under ROC curve is 0.661. T3-4 (OR=3.044, 95% CI 1.978-4.684, P<0.001), follicular variant of PTC (OR=0.034, 95% CI 0.174-0.530, P<0.001), ETE (OR=3.277, 95% CI 2.073-5.182, P<0.001), BRAFV600E mutation (OR=2.427, 95% CI 1.589-3.708, P<0.001) and trop-2 expression greater than cutoff point (OR=4.018, 95% CI 2.536-6.366, P<0.001) was a risk factor for LNM in univariate analysis. After adjusting, trop-2 expression (OR=2.068, 95% CI 1.032-4.142, P<0.001) and T3-4 (OR=5.126, 95% CI 2.344-11.210, P=0.04) was found to be an independent risk factor for LNM, which validated the diagnostic value of the cutoff point of trop-2 expression for LNM in FUSCC.

Overexpression of trop-2 is correlated with ETE in PTC

Whether overexpression of trop-2 was an independent risk factor for ETE in PTC was also analyzed. ROC analysis identified the best cutoff of trop-2 expression levels that were predictive of ETE was 0.479, area under ROC curve is 0.697. **Table 4** shows in FUSCC cohort, trop-2 expression greater than 0.479 (OR=4.611, 95% CI 2.103-10.108, P<0.001) was the only risk factor for ETE in univariate and multivariate analysis.

The best cutoff for trop-2 to predict ETE in TCGA cohort was -0.908, area under ROC curve is 0.66. **Table 5** shows age more than 55 years old (OR=2.036, 95% CI 1.293-3.206, P=0.002), follicular variant of PTC (OR=0.253, 95% CI 0.121-0.529, P<0.001), tall cell variant of PTC (OR=3.953, 95% CI 1.763-8.862, P=0.001), LNM (OR=3.277, 95% CI 2.073-5.182, P< 0.001), BRAFV600E mutation (OR=3.983, 95% CI 2.378-6.672, P<0.001) and trop-2 expression greater than 0.908 (OR=5.086, 95% CI 2.716-9.524, P<0.001) were a risk factors for ETE in univariate analysis. After adjusting, trop-2 expression (OR=2.778, 95% CI 1.344-5.742,

Table 5. Clinicopathologic and molecular factors associated with ETE in PTC in the TCGA cohort

Variables	Univariate analysis			Multivariate analysis		
	P-value	OR	95% CI for OR	*P*-value	OR	95% CI for OR
Female	0.907	1.030	0.623-1.703			
Age ≥55 years	0.002	2.036	1.293-3.206	0.001	2.442	1.465-4.071
Histological type						
Follicular PTC	<0.001	0.253	0.121-0.529	0.114	0.509	0.221-1.177
Tall-cell PTC	0.001	3.953	1.763-8.862	0.011	3.060	1.296-7.223
LNM	<0.001	3.277	2.073-5.182	<0.001	2.592	1.567-4.290
BRAFV600E	<0.001	3.983	2.378-6.672	0.185	2.201	0.685-7.073
Trop-2 (greater than cutoff)*	<0.001	5.086	2.716-9.524	0.006	2.778	1.344-5.742

Note: LNM, lymph node metastasis. *cutoff = -0.908.

P=0.006), age more than 55 years old (OR= 2.442, 95% CI 1.465-4.071, P=0.001), tall cell variant of PTC (OR=3.953, 95% CI tall cell variant of PTC (OR=3.953, 95% CI 1.763-8.862, P=0.001), LNM (OR=3.06, 95% CI 2.073-5.182, P<0.001), P=0.011), LNM (OR=2.592, 95% CI 1.567-4.290, P<0.001), were found to be independent risk factors for ETE, which validated the diagnostic value of trop-2 expression for ETE in FUSCC.

Overexpression of trop-2 is associated with BRAFV600E mutation

The positive rates of BRAFV600E mutation in the FUSCC and TCGA cohorts were 63.64% (63/99) and 59.42% (227/382), respectively. Trop-2 expression was associated with the BRAFV600E mutation status in PTC in the two cohorts (FUSCC: P<0.001, TCGA: P<0.001), showing a higher expression level in BRAFV600E-mutated PTC than the level in BRAFV600E wild-type PTC (**Table 1**). A multivariate logistic regression analysis was performed to investigate factors that could affect trop-2 expression in both the FUSCC and TCGA cohorts, and the outcomes indicated that BRAFV600E mutation was an independent risk factor with strong effect size (FUSCC: OR=4.084, 95% CI 1.675-9.957, P=0.002; TCGA: OR= 37.357, 95% CI 19.128-72.958, P<0.001, **Table 4**).

Discussion

PTC is a relatively indolent cancer which mostly can be controlled by the combination of surgical removal, radioactive ablation, and thyroxine, but not all are responsive to current therapy. Clinical decisions should be made based on the aggressiveness of individual tumor, that is, risk-stratification. For example, Japanese researchers have provided compelling data to support that active surveillance is a safe and effective alternative to immediate surgery for selected papillary microcarcinoma (PTMC) [14], but how to screen out the aggressive tumors is uncertain. Similar dilemmas include whether to do total thyroidectomy, prophylactic central neck dissection, and radioiodine ablation. Current risk stratification is mostly based on the clinical features and BRAFV600E mutation. BRAFV600E is the most common genetic mutation of PTC and is associated with aggressive behavior and recurrence rate of PTC, but its role as an independent risk factor is still controversial based on pooled data from multiple centers [15]. Thanks to next-generation sequencing technologies and programs like The Cancer Genome Atlas (TCGA), more genetic and epigenetic markers of PTC have been identified, but those molecules need to be further testified. In this study, we choose trop-2 because it is a molecule that has been rarely studied in PTC while it has been proved to be valuable in predicting prognosis in cancers from other origins. Also, previous studies had found trop-2 as an excellent biomarker of PTC in histology and cytology with the sensitivity and specificity no less than HBME-1 [3, 4]. Our study had not only confirmed those results with larger sample size, but also further investigated the possibility of using trop-2 as a biomarker of aggressiveness in PTC. We have collected a large amount of paired tissues from the patients of FUSCC and also the data from TCGA. In both cohorts, trop-2 was an independent risk factor for LNM and ETE which were the two major characteristics of aggressive behavior of cancer. In the

TCGA cohort, trop-2 expression is also associated with advanced T stage and TNM stage but not in the FUSCC cohort, but the ratio of high trop-2 expression in stage III (55%) and stage IV (61.1%) is higher, compared with stage I (47%) and stage II (50%), although it did not yield statistical significance. Since trop-2 is an independent risk factor of LNM and ETE, further analysis has been made to test its predictive value of LNM and ETE.

Moreover, our study also demonstrated that overexpression of trop-2 significantly correlated with BRAFV600E mutation. BRAFV600E and mutated RAS are two major driver mutations of PTC, tumors driven by BRAFV600E do not respond to the negative feedback from ERK to RAF, resulting in high MAPK-signaling. In contrast, tumors driven by RAS mutation respond to ERK feedback via RAF dimers resulting in lower MAPK-signaling. Integrated genomic characterization of PTC suggests that PTC could be divided into two subgroups based on molecular landscape: BRAFV600E-variant-like PTC and RAS-variant-like PTC. Our study found that trop-2 is relatively exclusive for BRAFV600E-variant-like PTCs, this indicate that trop-2 may be the downstream molecule of MAPK. Interestingly, previous studies reported that trop2 transduces an intracellular calcium signal which could activate the MAPK pathway. Based on these findings, the mechanism of interaction between trop-2 and MAPK pathway is not quite elucidated; further investigation should be conducted. Furthermore, in the group of patients without BRAF mutation, we found out that trop-2 was also associated with LNM but not ETE, which means that trop-2 could be used as a biomarker of LNM in the absence of BRAFV600E.

In recent years, clinical trials of systemic therapy like sorafenib [20] and lenvatinib [21] had demonstrated improvement of progression free survival in treating local advanced or radioiodine refractory PTC, but there is still no significant advantage in overall survival and quality of life, and novel drugs need to be explored. A series of drugs based on RS7 which is a humanized monoclonal antibody targeting trop-2 was developed. RS7 was used as a guide for radioactive isotopes or toxins. Sacituzumab govitecan is the conjugate of RS7 and SN-38 which is a topoisomerase I inhibitor that is the active metabolite of irinotecan [22]. It was conducted for several clinical trials of diverse metastatic solid tumors such as thyroid cancer, including pancreatic cancer, triple-negative breast cancer, colorectal cancer, and small cell lung cancer [23-25]. All of these trials had shown acceptable toxicity and encouraging therapeutic activity. Since overexpression of trop-2 also exists in PTC and is associated with the aggressive behavior, Sacituzumab govitecan and other trop-2 oriented drugs could offer more choices for those patients who failed first line therapy, especially when using it along with thyroxine, radioiodine therapy, and tyrosine kinase inhibitors. Investigations in pre-clinical and clinical studies could be warranted.

Conclusion

In summary, overexpression of trop-2 in PTC is correlated with LNM, ETE, and BRAF mutation, which makes it an excellent candidate as biomarker of aggressiveness of PTC and potential target of trop-2 oriented immunotherapy.

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Disclosure of conflict of interest

None.

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References

[1] La Vecchia C, Malvezzi M, Bosetti C, Garavello W, Betuccio P, Levi F, Negri E. Thyroid cancer mortality and incidence: a global overview. Int J Cancer 2015; 136: 2187-95.

[2] Kowalska A, Walczyk A, Kowalik A, Palyga I, Gasior-Perczak D, Trybek T, Kopczynski J, Kajor M, Mikina E, Szymonek M, Gadawska-Juszczyk K, Szyska-Skrobot D, Lizis-Kolus K, Hurej S, Chrapek M, chlopek M, Gozdz S. Response to therapy of papillary thyroid cancer of known

BRAF status. Cin Endocrinol (Oxf) 2017; 87: 815-824.
[3] Simms A, Jacob RP, Cohen C, Siddiqui MT. TROP-2 Expression in papillary thyroid carcinoma: potential diagnostic utility. Diagn Cytopathol 2016; 44: 26-31.
[4] Addati T, Achille G, Centrone M, Petroni S, Popescu O, Russo S, Grammatica L, Simone G. TROP-2 expression in papillary thyroid cancer: a preliminary cyto-histological study. Cytopathology 2015; 26: 303-311.
[5] Lipinski M, Parks DR, Rouse RV, Herzenberg LA. Human trophoblast cell-surface antigens defined by monoclonal antibodies. Proc Natl Acad Sci USA 1981; 78: 5147-5150.
[6] Ripani E, Sacchetti A, Corda D, Alberti S. Human Trop-2 is a tumor-associated calcium signal transducer. Int J Cancer 1998; 76: 671-676.
[7] Fornaro M, Dell'Arciprete R, Stella M, Bucci C, Nutni M, Capri MG, Alberti S. Cloning of the gene encoding Trop-2, a cell-surface glycoprotein expressed by human carcinomas. Int J Cancer 1995; 62: 610-618.
[8] Li Z, Jiang X, Zhang W. TROP2 overexpression promotes proliferation and invasion of lung adenocarcinoma cells. Biochem Biophys Res Commun 2016; 470: 197-204.
[9] Ohmachi T, Tanaka F, Mimori K, Inoue H, Yanaga K, Mori M. Clinical significance of TROP2 expression in colorectal cancer. Clin Cancer Res 2006; 12: 3057-3063.
[10] Trerotola M, Jernigan DL, Liu Q, Siddiqui J, Fatatis A, Languino LR. Trop-2 promotes prostate cancer metastasis by modulating β1 integrin functions. Cancer Res 2013; 73: 3155-3167.
[11] Iacobuzio-Donahue CA, Maitra A, Shen-Ong GL, van-Heek T, Ashfaq R, Walter K, Berg K, Hollingsworth MA, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH. Discovery of novel tumor markers of pancreatic cancer using global gene expression technology. Am J Pathol 2002; 160: 1239-1249.
[12] Cubas R, Zhang S, Li M, Chen C, Yao Q. Trop2 expression contributes to tumor pathogenesis by activating the ERK MAPK pathway. Mol Cancer 2010; 9: 253.
[13] Sobin LH, Gospodarowicz M, Wittekind C. UICC: TNM classification of malignant tumors. 7th edition. New York: Wiley-Liss; 2009.
[14] Ito Y, Miyauchi A, Inoue H, Fukushima M, Kihara M, Higashiyama T, Tomoda C, Takamura Y, Kobayashi K, Miya A. An observational trial for papillary thyroid microcarcinoma in Japanese patients. World J Surg 2010; 34: 28-35.
[15] Tufano RP, Teixeira GV, Bishop J, Carson KA, Xing M. BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. Medicine (Baltimore) 2012; 91: 274-286.
[16] Pratilas CA, Taylor BS, Ye Q, Viale A, Sander C, Solit DB, Rosen N. (V600E)BRAF is associated with disabled feedback inhibition of RAF-MEK signaling and elevated transcriptional output of the pathway. Proc Natl Acad Sci U S A 2009; 106: 4519-4524.
[17] Durante C, Puxeddu E, Ferretti E, Morisi R, Bruno R, Barbib F, Avenia N, Scipioni A, Verrienti A, Tosi E, CavaliereA, Gulino A, Filetti S, Russo D. BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab 2007; 92: 2840-2843.
[18] Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014; 159: 676-690.
[19] Cubas R, Zhang S, Li M, Chen C, Yao Q. Trop2 expression contributes to tumor pathogenesis by activating the ERK MAPK pathway. Mol Cancer 2010; 9: 253.
[20] Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, de la Fouchardiere C, Pacini F, Paschke R, Shong YK, Sherman SI, Smit JW, Chung J, Kappeler C, Pena C, Molnar I, Schlumberger MJ; DECISION investigators. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomized, double-blind, phase 3 trial. Lancet 2014; 384: 319-328.
[21] Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, Habra MA, Newbold K, Shah MH, Hoff AO, Gianoukakis AG, Kiyota N, Taylor MH, Kim SB, Krzyzanowska MK, Dutcus CE, de las Heras B, Zhu J, Sherman SI. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med 2015; 372: 621-630.
[22] Starodub AN, Ocean AJ, Shah MA, Guarino MJ, Picozzi VJ Jr, Vahdat LT, Thomas SS, Govindan SV, Maliakal PP, Wegener WA, Hamburger SA, Sharkey RM, Goldenberg DM. First-in-human trial of a novel Anti-Trop-2 antibody-SN-38 conjugate, sacituzumab govitecan, for the treatment of diverse metastatic solid tumors. Clin Cancer Res 2015; 21: 3870-3878.
[23] Bardia A, Mayer IA, Diamond JR, Moroose RL, Isakoff SJ, Starodub AN, Shah NC, O'Shaughnessy J, Kalinsky K, Guarino M, Abramson V, Juric D, Tolaney SM, Berlin J, Messersmith WA, Ocean AJ, Wegener WA, Maliakal P, Sharkey RM, Govindan SV, Goldenberg DM, Vahdat LT. Efficacy and safety of Anti-Trop-2 antibody drug conjugate sacituzumab govitecan (IMMU-132) in heavily pretreated patients with metastatic triple-negative breast cancer. J Clin Oncol 2017; 35: 2141-2148.

[24] Heist RS, Guarino MJ, Masters G, Purcell WT, Starodub AN, Horn L, Scheff RJ, Bardia A, Messersmith WA, Berlin J, Ocean AJ, Govindan SV, Maliakal P, Mudenda B, Wegener WA, Sharkey RM, Goldenberg DM, Camidge DR. Therapy of advanced non-small-cell lung cancer with an SN-38-Anti-Trop-2 drug conjugate, sacituzumab govitecan. J Clin Oncol 2017; 35: 2790-2797.

[25] Ocean AJ, Starodub AN, Bardia A, Vahdat LT, Isakoff SJ, Guarino M, Messersmith WA, Picozzi VJ, Mayer IA, Wegener WA, Maliakal P, Govindan SV, Sharkey RM, Goldenberg DM. Sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate for the treatment of diverse epithelial cancers: safety and pharmacokinetics. Cancer 2017; 123: 3843-3854.