Clinicopathologic Significance of TROP2 and Phospho-TROP2 in Gastric Cancer

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Abstract

Background: Trophoblast cell-surface antigen 2 (TROP2) is a transmembrane glycoprotein expressed in epithelial cells. TROP2 overexpression has been reported to be correlated with malignant progression in most carcinomas, but TROP2 showed a tumor-suppressive function in some types of cancers. We currently developed a novel antibody against phospho-TROP2 (pTROP2). Since the function of TROP2 is controversial, we then aimed to clarify the clinicopathologic significance of TROP2 and pTROP2 expression in human gastric cancer (GC) in this study.

Methods: We retrospectively analyzed the cases of 704 GC patients who underwent gastrectomy. The expressions of TROP2 and pTROP2 in each tumor were evaluated by immunohistochemistry. We analyzed the correlation between the GC patients' clinicopathologic features and the TROP2 and pTROP2 expression in their tumors.

Results: Overexpression of TROP2 and that of pTROP2 were identified in 330 (46.9%) and 306 (43.5%) of the 704 GC patients, respectively. TROP2 overexpression was significantly correlated with the histological intestinal type, high tumor invasion depth (T3/T4), lymph node metastasis, lymphatic invasion, and venous invasion. In contrast, pTROP2 overexpression was significantly correlated with intestinal type, low tumor invasion depth (T1/2), no lymph node metastasis, and no lymphatic invasion. TROP2 overexpression was significantly associated with poorer overall survival ($p < 0.01$, log rank), whereas pTROP2 overexpression was significantly associated with better overall survival ($p < 0.01$, log rank).

Conclusion: TROP2, but not pTROP2, might be associated with the metastatic ability of GC, resulting in poor prognoses for GC patients.

Background

There have been recent advances in the diagnostic and therapeutic techniques for gastric cancer (GC), but GC remains as the third leading cause of cancer mortality worldwide (1). Although adjuvant treatment has prolonged the survival of GC patients, the overall survival after surgery for GC remains poor (2, 3).

Trophoblast cell-surface antigen 2 (TROP2) encoded by *tumor-associated calcium signal transducer 2* (*TACSTD2*) gene is a transmembrane glycoprotein expressed in epithelial cells (4). TROP2 was identified in human trophoblast and choriocarcinoma cell lines (5). TROP2 was reported to bind to claudin1, claudin7, cyclin D1, protein kinase C (PKC), phosphatidylinositol 4,5-bisphosphate (PIP2), and insulin-like growth factor 1 (IGF1)(5–7). It is suspected that by its binding to these proteins, TROP2 might affect the tight junctions of epithelial cells (8), tumor proliferation (9), podosome formation, Raf and NF- κ B activation (5, 8), and IGF1 receptor (IGF1R) suppression (10).

The expression of TROP2 was described in several studies as being associated with the invasion and metastasis of cancer cells, resulting in poor prognoses of GC, pancreatic cancer, oral cancer, colon cancer, and ovarian carcinoma (11–16). However, TROP2 was reported to have a tumor-suppressive function in

cervical cancer, lung adenocarcinoma, and head and neck squamous cell cancer (10, 17, 18). The functions of TROP2 are thus a matter of controversy. In addition, there is no report about the clinicopathologic significance of phospho-TROP2 (pTROP2). We conducted the present study to clarify the clinicopathologic significance of TROP2 and pTROP2 in GC. This study is the first to reveal the correlation between the GC patients' clinicopathologic features and the TROP2 and pTROP2 expression in their tumors.

Methods

Patients

A total of 704 patients who were histologically confirmed to have primary GC was enrolled in this study. The patients underwent a resection of gastric tumor and regional lymph nodes. None of patients had undergone preoperative radiation and/or chemotherapy. The pathologic diagnoses and classifications were made according to the UICC TNM classification of malignant tumors. This study was approved by Osaka City University ethics committee (Reference number 924). Informed consent was obtained from all patients.

Immunohistochemistry of TROP2 and pTROP2

The expression of TROP2 and pTROP2 were evaluated by immunohistochemistry. The immunohistochemical determination of them were examined as the manufacturer's instructions. Briefly, Slides were deparaffinized and rehydrated with xylene and graded alcohol series and activated by heating. Endogenous peroxidase was blocked and then sections were incubated with an anti-mouse antibody for TROP2 (1:250, sc-376746, Santa Cruz Biotechnology) or anti-rabbit antibody for pTROP2 (1:200, obtained from Kyoto Sangyo University). Anti-rabbit antibody for pTROP2 was produced by the following method. Keyhole limpet hemocyanin (KLH) -conjugated peptides (Trop-2 cytoplasmic domain) with phosphorylated Ser-322 were emulsified with Freund's complete (first time) and incomplete (from second time) adjuvant and injected subcutaneously five times into a 12-week-old female New Zealand White rabbit (19). After the fifth immunization, blood was taken, and an IgG fraction was prepared from the serum by protein A-Sepharose column chromatography. The sections were incubated with biotinylated second antibody. They were treated with streptavidin-peroxidase reagent and counterstained with Mayer's hematoxylin. TROP2 expression was evaluated by intensity of staining and percentage of stained tumor cells. Intensity was given scores 0–3 (0: no, 1: weak, 2: moderate, 3: strong) and the percentage of stained tumor cells in all tumor cells was given scores 0–3 (0 = 0%, 1 = 1%-30%, 2 = 31%-70%, 3 = 71%-100%). The two scores were multiplied to obtain the final score of 0–9. TROP2 positive was defined as the score was 3 or more. pTROP2 expression was evaluated by intensity of staining of tumor cells and was given scores 0–3 like TROP2. pTROP2 positive was defined as the intensity score was 1 or more.

Statistical analysis

Statistical analysis was performed by R for Windows OS (version 3. 5. 2). The association between TROP2 or pTROP2 expression and clinicopathological variables were assessed by the chi-square test. Survival was measured from the date of surgery. Overall survival was analyzed by Kaplan-Meier method and compared by log-rank test. The Cox proportional hazards model was used for multivariate analysis. A p-value < 0.05 was considered as statistically significant.

Results

Immunostaining findings of TROP2 and pTROP2

Figure 1 provides representative immunostaining patterns of TROP2 and pTROP2. TROP2 and pTROP2 were stained at the cytoplasm and the cell membrane of cancer cells. TROP2 was expressed mainly at the cell membrane, whereas pTROP2 was expressed mainly at the cell cytoplasm. Of the total of 704 cases, 330 (46.9%) were TROP2-positive and 306 (43.5%) were pTROP2-positive.

The expressions of TROP2 and pTROP2 and their correlations with clinicopathological features

The clinicopathological features of all 704 patients based on the TROP2 and pTROP2 expression in their cancer cells are summarized in **Table I**. Compared to TROP2 negativity of cancer cells, TROP2 positivity of cancer cells was significantly associated with age > 60 years ($p < 0.01$), male gender ($p < 0.01$), differentiated type ($p < 0.01$), tumor depth (T3/T4) ($p < 0.01$), lymph node metastasis ($p < 0.01$), lymphatic invasion ($p < 0.01$), venous invasion ($p < 0.01$). The overexpression of pTROP2 was significantly correlated with differentiated type ($p < 0.01$), tumor depth (T1/T2) ($p < 0.01$), no lymph node metastasis ($p < 0.01$), and no lymphatic invasion ($p < 0.01$).

Survival

The 5-year overall survival (OS) rate of the 330 patients in the TROP2-positive group was significantly poorer compared to that of the TROP2-negative group ($p < 0.01$, Fig. 2A). The 5-year OS rate of the patients in the pTROP2-positive group was significantly better compared to that of the pTROP2-negative patients ($p < 0.01$, Fig. 2B). Our analysis by each tumor stage revealed that there was no significant difference in OS between the TROP2-positive and TROP2-negative cases at each tumor stage. The OS of the pTROP2-positive cases was not significantly different from that of the pTROP2-negative cases at each stage.

Univariate and multivariate analyses

The results of the univariate and multivariate analyses for OS are given in **Table II**. The univariate analysis showed that poor OS was significantly correlated with undifferentiated type ($p < 0.01$), depth of tumor (T3 and T4) ($p < 0.01$), lymph node metastasis ($p < 0.01$), distant metastasis ($p < 0.01$), venous invasion ($p < 0.01$), lymphatic invasion ($p < 0.01$), TROP2 overexpression ($p < 0.01$), and pTROP2 overexpression ($p < 0.01$). The multivariate analysis revealed that undifferentiated type ($p < 0.01$), depth of tumor ($p < 0.01$), lymph node metastasis ($p < 0.01$), and distant metastasis ($p < 0.01$) – but not TROP2 and pTROP2 – were significantly correlated with poorer OS.

Discussion

Our present analyses demonstrated that TROP2 overexpression was significantly associated with tumor depth, lymph node metastasis, and vessel invasion in GC. Stoyanova *et al.* reported that the intracellular domain of TROP2 might stimulate cyclin D1 and c-myc (20). TROP2 overexpression might be correlated with the progression of GC via up-regulations of cyclin D1 and c-myc. In the present patient series, the overall survival of the GC patients with TROP2 overexpression was poor. The univariate analysis indicated that the patients' overall survival was significantly correlated with TROP2, whereas the multivariate analysis demonstrated that TROP2 overexpression was not correlated with overall survival. These findings might indicate that TROP2's signal is associated with the progression of GC cells, and that TROP2 could be one of the predictive markers for poor survival of GC patients.

We observed herein that TROP2 overexpression was associated with the intestinal type of GC. Mühlmann *et al.* also reported that TROP2 was correlated with the histological intestinal type of GC (12). It has been reported that adhesion molecules such as claudins and cadherins might play an important role in the histology of cancer cells (21, 22). TROP2's signal up-regulates the tight junctions (which are associated with histologically intestinal type of GC), suggesting that TROP2 might be involved in the histological formation of GC.

In contrast, our analyses revealed that pTROP2 overexpression was associated with tumor depth (T1 or T2), no lymph node metastasis, and no lymphatic invasion, resulting in a good prognosis. The overexpression of pTROP2 might have tumor-suppressive functions with clinical significance that differs from that of TROP2. Sin *et al.* reported that TROP2 suppressed IGF1R and ALK signaling as a tumor-suppressing function (17). The mechanism of this suppression is that IGF1 and midkine bind to TROP2 and inhibit the signals of IGF1R and ALK, which play critical roles in cell growth, differentiation, transformation, and metastasis (23–25).

Mori *et al.* demonstrated that in colon cancer cells, PKC α and PKC δ were involved in TROP2 phosphorylation, and TROP2 phosphorylation changed the localization of claudin7 and promoted cell motility (19). TROP2 phosphorylation may have a suppressive effect on GC, and pTROP2 may inhibit the IGF1R and ALK signal pathway. The clinicopathologic significance of pTROP2 might differ among cancer types. Taken together, the above-described findings and our present results suggest that the phosphorylation of TROP2 may restrain tumor progression in GC.

Currently, several clinical trials using the therapeutic agents against TROP2, DS-1062 and IMMU-132, are ongoing in lung cancer (DS-1062, NCT 03401385), urothelial cancer (IMMU-132, NCT 03547973), and triple negative breast cancer (IMMU-132, NCT 04230109). It was reported that IMMU-132 had efficacy in a heavily pretreated population of patients with metastatic triple-negative breast cancer (26). Phase III study of IMMU-132 in patients with metastatic triple negative breast cancer is in progress (NCT 02574455). Our data suggest that a clinical trial using these agents might be useful for GC patients with TROP2 expression.

Conclusion

TROP2 might be associated with the tumor progression of GC cells, resulting in poor prognoses of patients with GC. pTROP2 might be associated with a tumor-suppressing function.

Abbreviations

TROP2: Trophoblast cell-surface antigen 2

pTROP2: phospho-Trophoblast cell-surface antigen 2

GC: gastric cancer

TACSTD2: tumor-associated calcium signal transducer 2

PKC: protein kinase C

PIP2: phosphatidylinositol 4,5-bisphosphate

IGF1: insulin-like growth factor 1

IGF1R: insulin-like growth factor 1 receptor

KLH: Keyhole limpet hemocyanin

OS: overall survival

Declarations

Ethics approval and consent to participate:

This study was approved by Osaka City University ethics committee (Reference number 924). Informed consent was obtained from all patients.

Consent for publication:

I agree to the publication.

Availability of data and materials:

The datasets used and analyzed during the current study are available from the first author and corresponding author on reasonable request.

Competing interests:

There are not any financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

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Authors' contributions:

Shuheï Kushiyaïa and Masakazu Yashiïo conceived of the presented idea. Shuheï Kushiyaïa performed the experiments and analyzed them. Shuheï Kushiyaïa and Masakazu Yashiïo verified the analytical methods. Masakazu Yashiïo supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Tables

Table I.

Correlation between the expression of TROP2 and pTROP2 in tumor cells and clinicopathologic features in 704 patients with gastric cancer.

	TROP2			pTROP2		
Clinicopathologic features	Negative (n=374)	Positive (n=330)	p Value	Negative (n=398)	Positive (n=306)	p Value
Age						
<60	134 (63.51%)	77 (36.49%)	<0.01	120 (56.87%)	91 (43.13%)	0.91
≥60	240 (48.68%)	253 (51.32%)		278 (56.39%)	215 (43.61%)	
Sex						
Female	183 (59.22%)	126 (40.78%)	<0.01	176 (56.96%)	133 (43.04%)	0.84
Male	191 (48.35%)	204 (51.65%)		222 (56.20%)	173 (43.80%)	
Microscopic type						
Differentiated	133 (41.82%)	185 (58.18%)	<0.01	147 (46.23%)	171 (53.77%)	<0.01
Undifferentiated	241 (62.44%)	145 (37.56%)		251 (65.03%)	135 (34.97%)	
Tumor depth						
T1, T2	207 (60.00%)	138 (40.00%)	<0.01	173 (50.14%)	172 (49.86%)	<0.01
T3, T4	167 (46.52%)	192 (53.48%)		225 (62.67%)	134 (37.33%)	
Lymph node metastasis						
Negative	225 (63.03%)	132 (36.97%)	<0.01	184 (51.54%)	173 (48.46%)	<0.01
Positive	146 (42.57%)	197 (57.43%)		211 (61.52%)	132 (38.48%)	
Lymphatic invasion						
Negative	181 (65.58%)	95 (34.42%)	<0.01	140 (50.72%)	136 (49.28%)	0.01
Positive	193 (45.20%)	234 (54.80%)		258 (60.42%)	169 (39.58%)	

Venous invasion						
Negative	330 (58.51%)	234 (41.49%)	<0.01	316 (56.03%)	248 (43.97%)	0.59
Positive	44 (31.43%)	96 (68.57%)		82 (58.57%)	58 (41.43%)	
Distant metastasis						
M0	358 (53.19%)	315 (46.81%)	0.99	380 (56.46%)	293 (43.54%)	0.7
M1	16 (53.33%)	14 (46.67%)		18 (60.00%)	12 (40.00%)	
pTROP2						
Negative	243 (61.06%)	155 (38.94%)	<0.01			
Positive	131 (42.81%)	175 (57.19%)				

Table II.
Univariate and multivariate Cox multiple regression analysis with respect to overall survival after surgery in patients with gastric carcinoma.

	Univariate analysis		Multivariate analysis	
	Hazard Ratio (95% CI)	p value	Hazard Ratio (95% CI)	p value
TROP2	1.56 (1.22 – 2.00)	<0.01	1.26 (0.96 - 1.66)	0.1
pTROP2	0.70 (0.54 - 0.90)	<0.01	0.86 (0.66 - 1.13)	0.28
Age $\geq$ 60 year-old	1.20 (0.91 - 1.58)	0.19		
Male (vs. Female)	1.18 (0.92 - 1.52)	0.19		
Undifferentiated type	1.93 (1.49 - 2.51)	<0.01	1.40 (1.06 – 1.86)	0.02
T3&4 (vs. T1&2)	8.26 (5.87 - 11.64)	<0.01	3.63 (2.43 - 5.42)	<0.01
N1-3 (vs. N0)	6.02 (4.43 - 8.18)	<0.01	2.35 (1.65 - 3.34)	<0.01
M1 (vs. M0)	5.42 (3.62 - 8.11)	<0.01	2.62 (1.74 – 3.94)	<0.01
Lymphatic invasion	5.60 (3.93 - 7.97)	<0.01	1.40 (0.92 - 2.13)	0.11
Vascular invasion	2.76 (2.13 - 3.59)	<0.01	1.26 (0.96 - 1.66)	0.1

Figures

Figure 1. Kushiyaama S. *et al.*

Figure 1

Representative pictures of TROP2 and pTROP2 expression of gastric cancer. TROP2 was expressed mainly at the cell membrane. pTROP2 was expressed mainly at the cell cytoplasm. A: A TROP2-negative case. B: A TROP2-positive case. C: A pTROP2-negative case. D: A pTROP2-positive case.

Figure 2. Kushiya S. *et al.*

Figure 2

The overall survival (OS) of gastric cancer patients based on TROP2 or pTROP2 expression. A: The Kaplan-Meier survival curve indicates that the OS of all of the patients with TROP2 overexpression was significantly worse than that of the patients with low TROP2 expression ($p < 0.01$). B: The Kaplan-Meier survival curve indicates that the OS of all of the patients with low pTROP2 expression in cancer cells was significantly worse than that of the patients with pTROP2 overexpression ($p < 0.01$).