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Does Trop2 Qualify as Prognostic Marker in Papillary RCC?

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Abstract

Background: Trophoblast cell surface antigen 2 (Trop2) is a transmembrane glycoprotein and promising biomarker for diagnosis and prognosis and a potential target for treatment. It plays a major role in signal transduction and is important in many aspects of tumorigenesis. Trop2 is a member of the Epithelial Cell Adhesion Molecule (EpCAM) family. It is overexpressed in several tumor types and associated with an aggressive phenotype. The prognostic role of Trop2 in Renal Cell Carcinoma (RCC), especially in papillary RCC (pRCC), is still unclear.

Patients and Methods: The patients' sample collection is a joint collaboration of the PANZAR consortium. Patients' medical history and tumor specimens were collected from n=240 and n=128 patients with type 1 and 2 pRCC, respectively. Expression of Trop2 was determined by immunohistochemistry.

Results: In total, Trop2 staining was positive in 55 of 240 type 1 and 30 of 128 type 2 pRCC cases. Kaplan-Meier analysis disclosed no significant difference in 5-year overall survival for all pRCC nor either subtype. However, in all pRCC Trop2 expression was found significantly more often in patients with a higher tumor stage (p=0.016) and advanced disease (p=0.015). Multivariate analysis could not identify Trop2 as an independent prognostic marker.

Conclusion: Trop2 was significantly associated with more advanced disease in all pRCC. However, Trop2 could not be identifying as an independent prognostic marker. Future studies are warranted to determine if Trop2 plays a role as prognostic marker in pRCC.

Keywords: Trop2; Papillary renal cell carcinoma; Prognosis; DAB; CIMP

Abbreviations

CIMP: CpG Island Methylator Phenotype; DAB: Diaminobenzidine; EGP-1: Epithelial Glycoprotein-1; EMT: Epithelial-to-Mesenchymal Transition; FH: Fumarate Hydratase; GA733–1: Gastric Antigen 733–1; HRP: Horseradish Peroxidase; IHC: Immunohistochemistry; MITF: Microphthalmia-Associated Transcription Factor; OS: Overall Survival; pRCC: Papillary Renal Cell Carcinoma; RCC: Renal Cell Carcinomas; TACSTD2: Tumor-Associated Calcium Signal Transducer 2; TMA: Tissue Micro Array; Trop2: Trophoblast Cell-Surface Antigen 2; WHO: World Health Organization

Introduction

Trophoblast cell-surface antigen 2 (Trop2) is a promising prognostic and diagnostic marker in several tumor entities. It is a cell-surface glycoprotein also known as TACSTD2 (Tumor-Associated Calcium Signal Transducer 2), GA733–1 (Gastric Antigen 733–1), and EGP-1 (Epithelial Glycoprotein-1). It is encoded by the gene TACSTD2 located on chromosome 1p32 [1]. An overexpression can be found in the majority of epithelial carcinomas, including abreast, urothelial, prostate, lung and pancreatic cancers [2]. An expression also can be detected in a variety of normal epithelial tissues such as liver, heart and kidney. The expression level is in these non-cancerous tissues much lower than the expression in epithelial tumors. This fact makes Trop2 a promising therapeutic target [3].

Despite growing data regarding Trop2, the function of this glycoprotein in tumor tissue remains unclear. For example, many recent studies suggest that Trop2 serves as mediator for metastasis and oncogene effects in prostate cancer *via* its regulation of focal adhesions and integrins [4]. On the other hand, Wang et al. demonstrated that a loss of Trop2 in mice triggers the development of squamous cell carcinoma with features of Epithelial-to-Mesenchymal Transition (EMT) [5]. This leads to the conclusion that the function of Trop2 depends on the tissue and the expression type, which makes the assessment more complex.

Papillary Renal Cell Carcinoma (pRCC) is the second most common tumor subtype of Renal Cell Carcinomas (RCC). The tumor comprises approximately 10% to 15% of all kidney neoplasms [6]. Up to now, it can be subdivided into two types, type 1 and 2, based on its morphological features. Type 1 tumors show a better clinical outcome than type 2 tumors. This typing system offers several problems in pathologically routine. Therefore, the upcoming World Health Organization (WHO) classification will change this system [7]. In addition, helpful prognostic biomarkers for this tumor type are still lacking.

Therefore, the aim of this study was to explore the prognostic role of Trop2 in pRCC. To the best of our knowledge, this is the first study addressing the expression and prognostic relevance of Trop2 in a large multicentre cohort of pRCC.

Patients and Methods

Patients and tumor characteristics

In total, 368 patients with papillary RCC, 240 (65.2%) type 1 and 128 (34.8%) type 2 were analyzed retrospectively. Specimen collection was a collaboration project of the PANZAR consortium. Contributing institutions were (in alphabetical order) Erlangen, Heidelberg, Herne, Homburg, Mainz, Mannheim, Marburg, Muenster, Munich (LMU) and Regensburg. Written informed consent from the patients were obtained by the participating institutions. The study was performed according to standards established in the Declaration of Helsinki. Renal surgery was performed between 1985 and 2007. After review by an experienced uropathologist (AH), one representative area of the pRCC tumors was selected to construct the tissue microarrays. For each case, the papillary subtype was defined according to 2004 WHO tumor classification. Pathological TNM grading according to 2002 TNM classification was performed.

Procedures

Expression of Trop2 was determined by Immunohistochemistry (IHC). Therefore, 2 µm TMA slides were stained for Trop2 (Anti-Trop2 antibody, ab214488, abcam, dilution 1:500). First of all, the antibody was applied for 30 min after heat pretreatment at 120°C for 5 min with Tris-EDTA buffer pH 9 and peroxidase blocking (Dako, Hamburg, Germany). The incubation with a Horseradish Peroxidase (HRP)-labeled secondary antibody polymer (EnVision, Dako) was conducted for 30 min. After that a Diaminobenzidine (DAB) substrate chromogen solution (Dako) was added for 10 min and counterstaining for 1 min with hematoxylin (Merck, Darmstadt, Germany). All incubation procedures were performed at room temperature. Positive controls and negative control slides without the addition of primary antibody were included for each staining experiment. Paraffin-embedded human colorectal cancer tissue was used as the positive control. All stained tissue samples were assessed in a blinded way by a pathologist (FE). For the evaluation we used a Leitz ARISTOPLAN light microscope (Leica Microsystems, Germany) with a x10 eyepiece, a 22-mm field of view and x40 objective lens (Plan FLUOTAR x40/0.70). The staining reaction was classified according to a semi-quantitative IHC reference scale as previously described [8,9]. Trop2 was localized primarily on the membrane and partly in the cytoplasm of tumor cells.

The staining intensity was scored from 0 to 3 (0= no staining, 1= weak staining, 2= moderate staining, 3= strong staining) according to the H-Score as already described [10-12]. The area of staining was evaluated in percent (0% to 100%), a staining intensity score was defined by multiplying the score with the stained area [13-15]. Given the absence of normative data on cell membrane or cell cytoplasm staining intensity in the literature, values in our patient collective were dichotomized using the median of observed distribution as the cut off. Because of the limited number of cases a binary cutoff was used.

A Trop2 staining lower or equal to the median was defined as Trop2 negative, and a staining higher than the median was defined as Trop2 positive.

Statistical analysis

The primary endpoint of the study was Overall Survival (OS). In the absence of death, the endpoint was censored at the date of last follow-up. The duration of follow-up was calculated from the date of surgery to the date of death or last known follow-up. Dependent upon the nature of variable, Chi-square, Fisher's exact tests, Mann-Whitney U-Test, or independent t-test were used as appropriate, to compare between patient/tumor characteristics and the corresponding subgroup with or without Trop2 expression. Kaplan-Meier survival times were estimated, with subgroups being compared using the log-rank test. Afterwards we performed Cox regression for all pRCCs with survival status as dependent variable, OS in

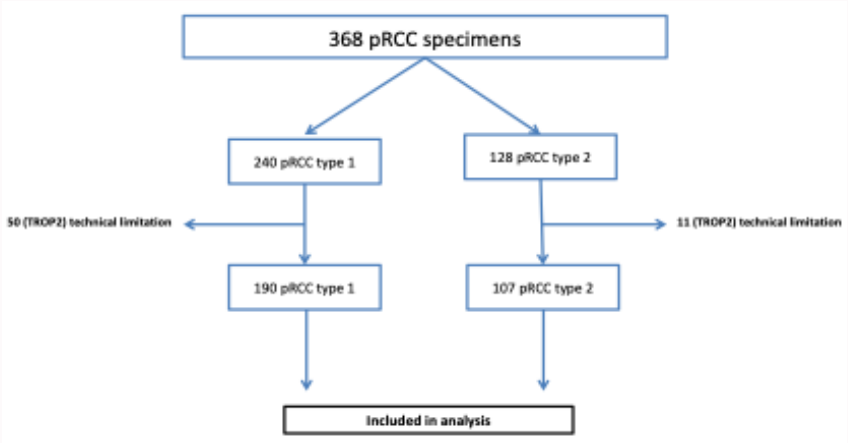

Figure 1: Study flow chart.
pRCC: papillary Renal Cell Carcinoma, number of patients included

months and Trop2 status, age, sex, T status, metastasis, lymph node metastasis, and grade as independent variables. SPSS 27.0 (USA) was used for statistical assessment. Two-sided p-values below 0.05 were considered statistically significant.

Results

Trop2 staining was evaluable in 190 of 240 patients with pRCC type 1 and in 107 of 128 patients with pRCC type 2 (Figure 1). The patients' clinicopathological characteristics are presented in Table 1.

Trop2 expression pattern in pRCC type 1

In total, Trop2 staining was positive in 86 (45.3%) of type 1 pRCC specimens. We could not find an association between Trop2 expression with neither patient nor tumor characteristics (Table 2).

Trop2 expression pattern in pRCC type 2

In total, Trop2 staining was positive in 59 (55.1%) of type 2 pRCC specimens. Univariant analysis showed no significant difference for in age, sex, grade, tumor stage, lymph node/distant metastasis or advanced for patients with Trop2+ tumors (Table 2).

Trop2 expression pattern in all pRCCs

Univariant analysis showed no significant difference for in age, sex, grade for patients with Trop2+ tumors. Interestingly, patients with Trop2+ tumors showed higher tumor stages (Chi square, p=0.016) and had more often an advanced disease (Fisher exact, p=0.015) compared to patients without Trop2- tumors). Multivariate analysis identified age, higher tumor stage and distant metastasis as independent predictor of OS in patients with pRCC but not Trop2 expression.

Trop2 expression and clinical course in type 1 pRCC

Median follow-up was 49.0 (IQR 25.0-84.5) months. At the time of last follow-up, 73 (70.2) and 51 (59.3%) patients were alive, 11 (10.6%) and 16 (18.6%) patients had died and 20 (19.2%) and 19 (22.1%) patients were lost to follow up in the Trop2- *vs.* Trop2+ subgroups respectively (p=0.204, chi square). Kaplan-Meier analysis disclosed a 5-year OS of 87.7% for patients with Trop2- tumors compared to 77.0% for patients with Trop2+ tumors in pRCC type 1 (p=0.102, log-rank; Figure 3a).

Trop2 expression and clinical course in type 2 pRCC

Median follow-up was 29 (IQR 18.0-71.8) months. At the time of last follow-up, 23 (47.9) and 28 (47.5%) were alive, 13 (27.1) and 15 (25.4%) patients had died and 12 (25.0) and 16 (27.1%) patients were lost to follow up in the Trop2- and Trop2+ subgroups respectively (p=0.963, chi square). Kaplan-Meier analysis disclosed a 5-year OS for patients with Trop2- tumors of 64.8% compared to 58.8% in patients with Trop2+ pRCC type 2 tumors compared to (p=0.754, log-rank; Figure 3b).

Trop2 expression and clinical course in the total sample

Median follow-up was 42.0 (IQR 21.0-81.3) months. At the time of last follow-up, 96 (63.2 %) and 79 (54.5 %) patients were alive, 24 (15.8%) and 31 (21.4%) patients had died and 32 (21.1%) and 35 (24.1%) patients were lost to follow up in the Trop2- and Trop2+ subgroups respectively (p=0.285, chi square). Kaplan-Meier analysis disclosed a 5-year OS of 84.2% for patients with Trop2- tumors compared to 77.0% for patients with Trop2+ tumors in pRCC type 1 (p=0.185, log-rank; Figure 2).

Discussion

As already mentioned above, pRCC is actually subdivided into two groups, type 1 and 2, based on its morphological features. Several studies demonstrated that type 2 tumors show a worse clinical outcome than type 1 tumors [16]. Moreover, the tumor subtypes are characterized by specific genetic alterations. *MET* alterations are associated with Type 1 tumors. Type 2 tumors are more complex and could be classified into three individual subgroups based on molecular differences [17]. In type 2 tumors several genetic alterations can be found: *CDKN2A* silencing, *SETD2* mutations, *TFE3* fusions, and increased expression of the NRF2-ARE pathway. Furthermore, in a subgroup of type 2 tumors a CpG Island Methylator Phenotype (CIMP) was detected [17]. This subtype is characterized by poor survival and mutation of the *Fumarate Hydratase* (*FH*) gene. Despite the clinically and biologically distinct of both tumor types the previous division into type 1 and type 2 subtypes will not be recommended in the future [18]. The most important reasons are poor interobserver reproducibility and the lack of proven clinical significance. In the pathological routine a relevant proportion of tumors demonstrate overlapping features [19]. Furthermore, type 2 tumors are very heterogenous tumors with several genetic alterations. Therefore, several new uncommon entities have already been defined, which also can be found in type 2 tumors. The group of type 2 pRCC includes for example FH-deficient RCC and Microphthalmia-Associated

Table 1: pRCC type 1 and type 2 patients' and tumor characteristics.

Variable	pRCC total *n=297*	pRCC type 1 *n*=190 (67.80%)	pRCC type 2 *n*=107 (32.20%)	p
Age [a], median (IQR) years	63.3 (55.0-71.0)	63.0 (54.2-70.0)	66.0 (57.0 – 73.1)	0.102[b]
Sex				0.741[c]
Female, *n* (%)	52 (17.5)	33 (17.4)	19 (17.8)	
Male, *n* (%)	190 (64.0)	127 (66.8)	63 (58.9)	
NE, *n* (%)	55 (18.5)	30 (15.8)	25 (23.4)	
T-stage				<0.001[d]
pT1, *n* (%)	142 (47.8)	106 (55.8)	36 (33.6)	
pT2, *n* (%)	51 (17.2)	37 (19.5)	14 (13.1)	
pT3, *n* (%)	46 (15.5)	17 (8.9)	29 (27.1)	
pT4, *n* (%)	1 (0.3)	0 (0.0)	1 (0.9)	
pTx, *n* (%)	57 (19.2)	30 (15.8)	27 (25.2)	
Grade				<0.001[d]
G1, *n* (%)	48 (16.2)	48 (25.3)	0 (0.0)	
G2, *n* (%)	125 (42.1)	106 (55.8)	19 (17.8)	
G3, *n* (%)	85 (28.6)	19 (10.0)	66 (61.7)	
Gx, *n* (%)	39 (13.1)	17 (8.9)	22 (20.6)	
N metastasis[a]				<0.001[c]
N-, *n* (%)	274 (92.3)	184 (96.8)	90 (84.1)	
N+, *n* (%)	23 (7.7)	6 (3.2)	17 (15.9)	
M Metastasis[a]				<0.001[c]
M-, *n* (%)	218 (73.4)	155 (81.6)	63 (58.9)	
M+, *n* (%)	16 (5.4)	2 (1.1)	14 (13.1)	
Mx, *n* (%)	63 (21.2)	33 (17.4)	30 (28.0)	
Locally or advanced				<0.001[c]
pT1/pT2 N0 M0, n (%)	187 (63.0)	140 (73.7)	47 (43.9)	
pT3/pT4 and/or N1 and/or M1, n (%)	48 (16.2)	18 (9.5)	30 (28.0)	
NE, n (%)	62 (20.9)	32 (16.8)	30 (28.0)	
Variable	**pRCC total** *n*=297	**pRCC type 1** *n*=190 (67.80%)	**pRCC type 2** *n*= 107 (32.20%)	**p**
Age [a], median (IQR) years	63.3 (55.0-71.0)	63.0 (54.2-70.0)	66.0 (57.0 – 73.1)	0.102[b]
Sex				0.741[c]
Female, *n* (%)	52 (17.5)	33 (17.4)	19 (17.8)	
Male, *n* (%)	190 (64.0)	127 (66.8)	63 (58.9)	
NE, *n* (%)	55 (18.5)	30 (15.8)	25 (23.4)	
T-stage				<0.001[d]
pT1, *n* (%)	142 (47.8)	106 (55.8)	36 (33.6)	
pT2, *n* (%)	51 (17.2)	37 (19.5)	14 (13.1)	
pT3, *n* (%)	46 (15.5)	17 (8.9)	29 (27.1)	
pT4, *n* (%)	1 (0.3)	0 (0.0)	1 (0.9)	
pTx, *n* (%)	57 (19.2)	30 (15.8)	27 (25.2)	
Grade				<0.001[d]
G1, *n* (%)	48 (16.2)	48 (25.3)	0 (0.0)	
G2, *n* (%)	125 (42.1)	106 (55.8)	19 (17.8)	
G3, *n* (%)	85 (28.6)	19 (10.0)	66 (61.7)	
Gx, *n* (%)	39 (13.1)	17 (8.9)	22 (20.6)	
N metastasis[a]				<0.001[c]

N-, *n* (%)	274 (92.3)	184 (96.8)	90 (84.1)	
N+, *n* (%)	23 (7.7)	6 (3.2)	17 (15.9)	
M Metastasis[a]				<0.001[c]
M-, *n* (%)	218 (73.4)	155 (81.6)	63 (58.9)	
M+, *n* (%)	16 (5.4)	2 (1.1)	14 (13.1)	
Mx, *n* (%)	63 (21.2)	33 (17.4)	30 (28.0)	
Locally or advanced disease				<0.001[c]
pT1/pT2 N0 M0, n (%)	187 (63.0)	140 (73.7)	47 (43.9)	
pT3/pT4 and/or N1 and/or M1, n (%)	48 (16.2)	18 (9.5)	30 (28.0)	
NE, n (%)	62 (20.9)	32 (16.8)	30 (28.0)	

pRCC type 1 and type 2 patient's and tumor characteristics of specimens eligible for Trop2 IHC staining. **Legend:** ([a]) at time of renal surgery; LN: Lymph Node; NE: Not Evaluable; N-: Lymph Node status unknown or tumor cells absent from regional lymph nodes; N+: Regional Lymph Node metastasis present; M-: No Evidence of Metastatic disease; M+: Evidence of Metastatic disease; ([b]) Mann-Whitney-U Test, ([c]) Fisher Exact test; ([d]) Chi square test

Table 2: pRCC patient's and tumor characteristics in dependence of Trop2 expression.

Variable	pRCC type 1 Trop2$^-$ *n*=104 (54.70%)	pRCC type 1 Trop2$^+$ *n*=86 (45.30%)	*p*-value	pRCC type 2 Trop2$^-$ *n*=48 (44.90%)	pRCC type 2 Trop2$^+$ *n*=59 (55.10%)	*p*-value
Age[a], median (IQR) years	61.0 (52.8-68.6)	64.0 (56.8-72.0)	0.057[b]	63.9 (55.0-73.9)	66.0 (57.0-72.5)	0.681[b]
Sex			0.518[c]			1.0[c]
Female, *n* (%)	20 (19.2)	13 (15.1)		28 (58.3)	11 (18.6)	
Male, *n* (%)	69 (66.3)	58 (67.4)		28 (58.3)	35 (59.3)	
NE, *n* (%)	15 (14.4)	15 (17.4)		12 (25.0)	13 (22.0)	
T-stage			0.083[d]			0.210[d]
pT1, *n* (%)	65 (62.5)	41 (47.7)		20 (41.7)	16 (27.1)	
pT2, *n* (%)	18 (17.3)	19 (22.1)		4 (8.3)	10 (16.9)	
pT3, *n* (%)	6 (5.8)	11 (12.8)		11 (22.9)	18 (30.5)	
pT4, *n* (%)	0 (0.0)	0 (0.0)		0 (0.0)	1 (1.7)	
pTx, *n* (%)	15 (14.4)	15 (17.4)		13 (27.1)	14 (23.7)	
Grade			0.177[d]			0.796[d]
G1, *n* (%)	29 (27.9)	19 (22.1)		0 (0)	0 (0)	
G2, *n* (%)	62 (59.6)	44 (51.2)		8 (16.7)	11 (18.6)	
G3, *n* (%)	7 (6.7)	12 (14.0)		30 (62.5)	36 (61.0)	
Gx, *n* (%)	6 (5.8)	11 (12.8)		10 (20.8)	12 (20.3)	
LN metastasis[a] Or N stage			0.413[c]			0.436[c]
N-, *n* (%)	102 (98.1)	82 (95.3)		42 (87.5)	48 (81.4)	
N+, *n* (%)	2 (1.9)	4 (4.7)		6 (12.5)	11 (18.6)	
Distant Metastasis[a] Or M stage			0.504[c]			*0.566*[c]
M-, *n* (%)	86 (82.7)	69 (80.2)		26 (54.2)	37 (62.7)	
M+, *n* (%)	2 (1.9)	0 (0.0)		7 (14.6)	7 (11.9)	
Mx, *n* (%)	16 (15.4)	17 (19.8)		15 (31.3)	15 (25.4)	
Locally or advanced			0.139[c]			0.239[c]
pT1/pT2 N0 M0, n (%)	181 (77.9)	59 (68.6)		23 (47.9)	24 (40.7)	
pT3/pT4 and/or N1 and/or M1, n (%)	7 (6.7)	11 (12.8)		10 (20.8)	20 (33.9)	
NE, n(%)	16 (15.4)	16 (18.6)		15 (31.3)	15 (25.4)	

Legend: ([a]) at time of renal surgery; LN: Lymph Node; NE: Not Evaluable; N-: Lymph Node status unknown or tumor cells absent from regional lymph nodes; N+: Regional Lymph Node metastasis present; M-: No Evidence of Metastatic diseases; M+: Evidence of Metastatic disease; ([b]) Mann-Whitney-U Test; ([c]) Fisher Exact test; ([d]) Chi square test

Transcription Factor (MITF) family translocation RCC. Hence, the type 1 and 2 classification for pRCC will not be recommended in the upcoming WHO classification [20].

Therefore, we evaluated the prognostic effect of Trop2 in pRCC in general and type-specific pRCC. In the type-specific analysis we found no significant associations regarding OS. However, if patients

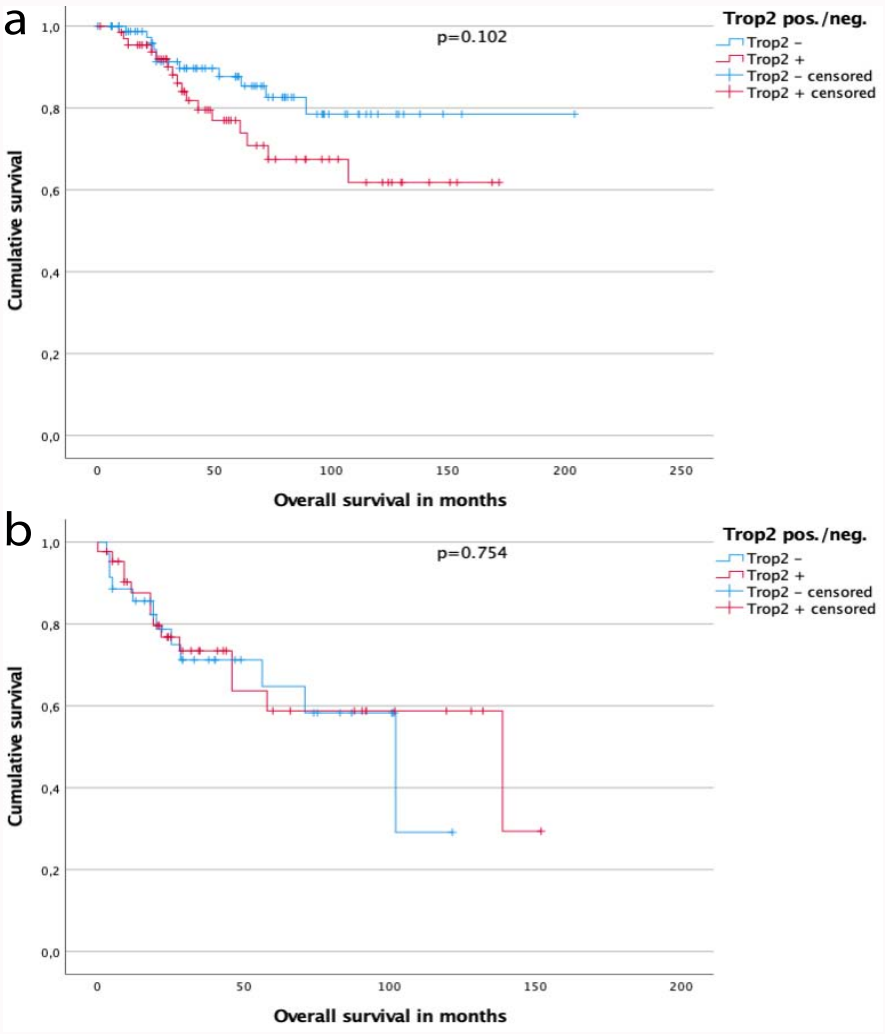

Figure 2: Kaplan-Meier analysis disclosed a 5-year OS of 87.7% for patients with Trop2- tumors compared to 77.0% for patients with Trop2+ tumors in pRCC type 1 (p= 0.102, log-rank) (2a). Kaplan-Meier analysis disclosed a 5-year OS for patients with Trop2 - tumors of 64.8% compared to 58.8% in patients with Trop2 + pRCC type 2 tumors compared to (p=0.754, log-rank) (2b).

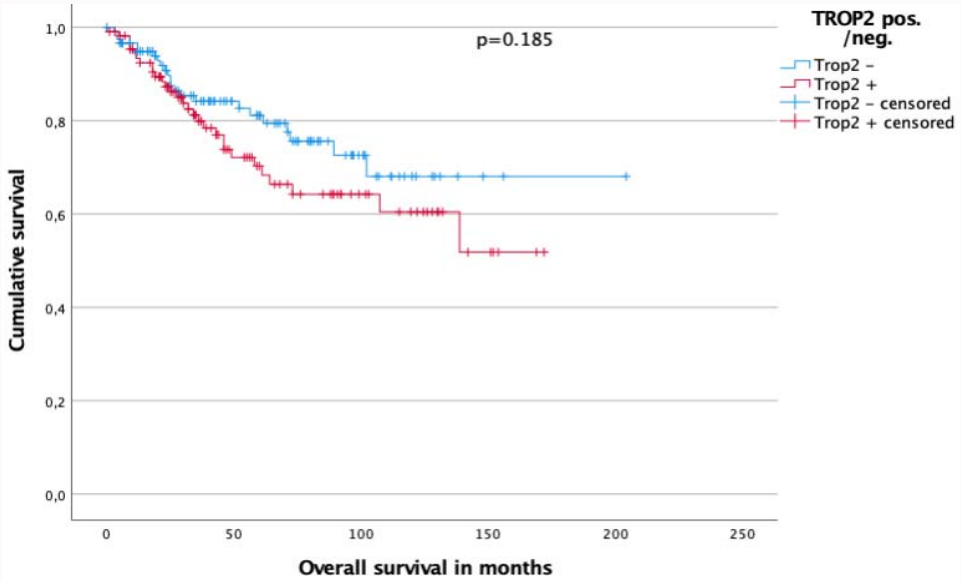

Figure 3: Kaplan-Meier analysis disclosed a 5-year OS of 84.2 % for patients with Trop2 - tumors compared to 77.0% for patients with Trop2 + tumors in all types pRCC (p=0.185, log-rank).

Table 3: pRCC patient's and tumor characteristics in dependence of Trop2 expression.

Variable	pRCC Trop2$^-$ *n*=152 (51.20%)	pRCC Trop2$^+$ *n*=145 (48.80%)	*p*-value
Age[a], median (IQR) years	63.0 (53.7 -69.3)	65.0 (57.0 -72.0)	0.054[b]
Sex			0.756[c]
Female, *n* (%)	28 (18.4)	24 (16.6)	
Male, *n* (%)	97 (63.8)	93 (64.1)	
NE, *n* (%)	27 (17.8)	28 (19.3)	
T-stage			**0.016**[d]
pT1, *n* (%)	85 (55.9)	57 (39.3)	
pT2, *n* (%)	22 (14.5)	29 (20.0)	
pT3, *n* (%)	17 (511.2)	29 (20.0)	
pT4, *n* (%)	0 (0.0)	1 (0.7)	
pTx, *n* (%)	28 (18.4)	29 (20.0)	
Grade			0.102[d]
G1, *n* (%)	29 (19.1)	19 (13.1)	
G2, *n* (%)	70 (46.1)	55 (37.9)	
G3, *n* (%)	37 (24.3)	48 (33.1)	
Gx, *n* (%)	16 (10.5)	23 (15.9)	
LN metastasis[a]			0.129[c]
N-, *n* (%)	144 (94.7)	130 (89.7)	
N+, *n* (%)	8 (5.3)	15 (10.3)	
Metastasis[a]			0.799[c]
M-, *n* (%)	112 (73.7)	106 (73.1)	
M+, *n* (%)	9 (5.9)	7 (4.8)	
Mx, *n* (%)	31 (20.4)	32 (22.1)	
Locally or advanced disease			**0.015**[c]
pT1/pT2 N0 M0, n (%)	104 (68.4)	83 (57.2)	
pT3/pT4 and/or N1 and/or M1, n (%)	17 (11.2)	31 (121.4)	
NE, n (%)	31 (20.4)	31 (21.4)	

Legend: ([a]) at time of renal surgery; NE: Not Evaluable; N-: Lymph Node status unknown or tumor cells absent from regional lymph nodes; N+: Regional Lymph Node metastasis present; M-: No Evidence of Metastatic diseases; M+: Evidence of Metastatic disease; ([b]) Mann-Whitney-U Test; ([c]) Fisher Exact test; ([d]) Chi square test

with pRCC type 1 and 2 are analyzed together, we detected that Trop2 expression was significantly higher in patients with a higher tumor stage and advanced disease. This result could give a first indication that Trop2 is associated with higher tumor mass, which could be an indirect prognostic marker for this entity. The prognostic relevance of Trop2 has already been described in several tumor entities, for example in colorectal cancer, in pancreatic cancer and in hepatocellular carcinoma [20-22]. Future studies will have to determinate whether Trop2 could serve as a prognostic marker in pRCC.

Of course, our study has several limitations, which should be mentioned: Beside the limited number of cases due to the incidence of this tumor type, there are some methodical limitations to mention. On the one hand the methodology of immunohistochemistry and the interpretation system. On the other the use of TMAs, as well as the use of retrospective analysis.

In summary, this is the first study addressing the expression and

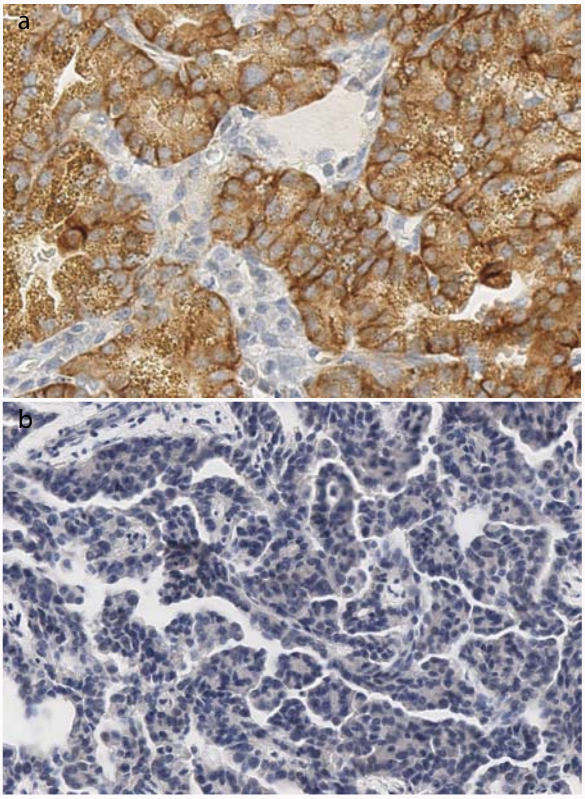

Figure 4: Immunohistochemical staining of Trop2 in pRCC specimen. (a) Positive (40x magnification) (b) Negative (40x magnification).

prognostic relevance of Trop2 in a large multicentre cohort of pRCC. Our results demonstrate that Trop2 was significantly associated with more advanced disease in all pRCC. However, Trop2 could not be identifying as an independent prognostic marker. Future studies are warranted to determine if Trop2 plays a role as prognostic marker in pRCC.

Author's Contribution

FE and SS participated in the data interpretation and drafting of the manuscript. MM and SS performed the statistical analysis. EH took great part in starting the collective material for this study and clinical data acquisition. AH, AA, FE, CS and IP carried out pathological data acquisition. CS, IP and AH constructed the tissue micro arrays. FE carried out the IHC evaluation. All others participated in collecting the material and clinical data acquisition. SS coordinated the project. All authors contributed to data interpretation and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethical Standards

Patient sample collection was a joint collaboration of the PANZAR consortium. All procedures have been performed in accordance with the at the time valid ethical standards and according to the 1964 Declaration of Helsinki and its later amendments. Informed consent was assessed prior to intervention. Details that disclose the identity of the subjects under study were omitted.

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