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TRPS1 is a Highly Sensitive Marker for Breast Cancer

A Tissue Microarray Study Evaluating More Than 19,000 Tumors From 152 Different Tumor Entities

Maximilian Lennartz, MD, Neele Löhr,* Doris Höflmayer, MD,* Sebastian Dwertmann Rico, MD,* Clara von Bargen, MD,* Simon Kind, MD,* Viktor Reiswich, MD,* Florian Viehweger, MD,* Florian Lutz, MD,* Veit Bertram, MD,* Christoph Fraune, MD,* Natalia Gorbokon, MD,* Sören Weidemann, MD,* Niclas C. Blessin, MD,* Claudia Hube-Magg, PhD,* Anne Menz, MD,* Ria Schlichter, MD,* Till Krech, MD,*† Andrea Hinsch, MD,* Eike Burandt, MD,* Guido Sauter, MD,* Ronald Simon, PhD,* Martina Kluth, PhD,* Andreas H. Marx, MD,‡ Patrick Lebok, MD,*† David Dum, MD,* Sarah Minner, MD,* Frank Jacobsen, MD,* Till S. Clauditz, MD,* Christian Bernreuther, MD,* and Stefan Steurer, MD**

Abstract: Trichorhinophalangeal syndrome 1 (TRPS1) is a nuclear protein highly expressed in breast epithelial cells. TRPS1 immunohistochemistry (IHC) has been suggested as a breast cancer marker. To determine the diagnostic and prognostic utility of TRPS1 IHC, tissue microarrays containing 19,201 samples from 152 different tumor types and subtypes were analyzed. GATA3 IHC was performed in a previous study. TRPS1 staining was seen in 86 of 152 tumor categories with 36 containing at least one strongly positive case. TRPS1 staining predominated in various types of breast carcinomas (51%-100%), soft tissue tumors (up to 100%), salivary gland tumors (up to 46%), squamous cell carcinomas (up to 35%), and gynecological cancers (up to 40%). TRPS1 positivity occurred in 1.8% of 1083 urothelial neoplasms. In invasive breast carcinoma of no special type, low TRPS1 expression was linked to high grade ($P = 0.0547$), high pT ($P < 0.0001$), nodal metastasis ($P = 0.0571$), loss of estrogen receptor and progesterone receptor expression ($P < 0.0001$ each), and triple-negative status ($P < 0.0001$) but was unrelated to patient survival ($P = 0.8016$). In squamous cell carcinomas from 11 different sites, low TRPS1 expression was unrelated to tumor phenotype. Positivity for both TRPS1 and GATA3 occurred in 47.4% to 100% of breast cancers, up to 30% of salivary gland tumors, and 29 (0.3%) of 9835 tumors from 134 other cancer entities. TRPS1 IHC has high utility for the identification of cancers of breast (or salivary gland) origin, especially in combination with GATA3. The virtual absence of TRPS1 positivity in urothelial neoplasms is useful for the distinction of GATA3-positive urothelial carcinoma from breast cancer.

Key Words: TRPS1, tissue microarray, immunohistochemistry, diagnostic marker, breast cancer

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From the *Department of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; †Department of Pathology, Clinical Center Osnabrueck, Osnabrueck, Germany; and ‡Department of Pathology, Academic Hospital Fuerth, Fuerth, Germany.

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Correspondence: Ronald Simon, PhD, Institute of Pathology, University Medical Center Hamburg-Eppendorf, Martinistr 52, Hamburg 20246, Germany (e-mail: r.simon@uke.de).

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Trichorhinophalangeal syndrome 1 (TRPS1), also termed transcriptional repressor GATA binding 1, is an atypical GATA nuclear transcription factor that mainly acts as a transcriptional repressor protein that can prevent the expression of GATA1-6-regulated genes (summarized[1]). For example, Runx1 and Sox9, which both are GATA3 regulated and required for cartilage formation, are suppressed by TRPS1.[2,3] Multiple germline mutations of the *TRPS1* gene were found to cause craniofacial and

skeletal malformations.[4] TRPS1 can also induce the expression of cancer-related genes, such as, for example, *FOXA1*, which is a negative regulator of epithelial-mesenchymal transition.[5] Functional studies in cancer cells have suggested a tumor-suppressive activity of TRPS1 by preventing epithelial-mesenchymal transition,[5,6] as well as a role of TRPS1 in breast cancer angiogenesis[7] and in multidrug resistance of breast cancer[8] and osteosarcoma.[9]

In normal tissues, TRPS1 is expressed in numerous cell types but expression is highest in breast epithelial cells (https://www.proteinatlas.org/ENSG00000104447-TRPS1). Because TRPS1 expression is often retained in cells that undergo malignant transformation, TRPS1 immunohistochemistry (IHC) has been suggested to represent a useful tool for the distinction of breast cancer from other cancer types in metastatic tissue.[10,11] However, TRPS1 expression has recently also been described in various salivary gland tumors,[10,12–14] endometrial carcinoma,[15] hepatocellular carcinoma,[16] colorectal cancer,[17] gastric cancer,[18] squamous cell carcinoma of the skin,[19] synovial sarcoma,[20] and osteosarcoma,[21] as well as in lung[22] and prostate cancer cell lines.[23,24] In carcinomas of the breast,[25] colon,[17] and stomach,[18] as well as in osteosarcoma,[21] TRPS1 expression levels were associated with parameters of cancer aggressiveness. Altogether, the available data suggest a diagnostic and prognostic potential of TRPS1 IHC analysis which may be enhanced by a combination with GATA3, another commonly used breast cancer marker.[10,26,27] However, many tumor entities have, so far, not been examined for TRPS1 by IHC and a systematic evaluation of different tumor entities for TRPS1 protein expression is so far lacking.

To better understand the prevalence and potential role of TRPS1 protein expression in tumors and to elucidate the potential diagnostic utility of TRPS1 IHC alone and in combination with GATA3, a survey of TRPS1 immunostaining in a broad range of tumor types is needed. In this study, we, therefore, evaluated TRPS1 expression in more than 19,000 tumor samples from 152 tumor types and subtypes for which GATA3 data were available in a tissue microarray (TMA) format.

MATERIALS AND METHODS

Tissue Microarrays

Our normal tissue TMA was composed of 8 samples from 8 different donors for each of 76 different normal tissue types (608 samples on one slide). The tumor TMAs contained a total of 19,201 primary tumors from 152 tumor types and subtypes. Detailed histopathological data on grade, pathologic tumor stage, pathologic lymph node status, and molecular data were available from 3173 tumors (1680 invasive breast carcinomas of no special type, 40 endometrioid and 369 serous ovarian carcinomas, 182 endometrioid endometrium carcinomas, and 902 squamous cell carcinomas). Clinical follow-up data were available from 877 invasive breast carcinomas of no special type with a median follow-up time of 49 months (range: 1 to 88 mo). Data on GATA3 were available for 15,964 tumors from an earlier study evaluating a large subset of our TMAs.[28] The composition of both normal and tumor TMAs is described in detail in the results section. Samples were from the archives of the Institutes of Pathology, University Hospital of Hamburg, Germany, the Institute of Pathology, Clinical Center Osnabrueck, Germany, and Department of Pathology, Academic Hospital Fuerth, Germany. Tissues were fixed in 4% buffered formalin and then embedded in paraffin. TMA tissue spot diameter was 0.6 mm. The use of archived remnants of diagnostic tissues for manufacturing of TMAs and their analysis for research purposes, as well as patient data analysis, has been approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics Commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

Immunohistochemistry

Freshly cut TMA sections were immunostained on 1 day and in 1 experiment. Slides were deparaffinized with xylol at room temperature for 3×5 minutes, rehydrated through a graded alcohol series, and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C in pH 7.8, Dako Target Retrieval Solution (Agilent; #S2367). Endogenous peroxidase activity was blocked with Dako Peroxidase Blocking Solution (Agilent; #52023) for 10 minutes. A primary antibody specific for TRPS1 (rabbit recombinant monoclonal, MSVA-512R, MS Validated Antibodies; 5676-512R) was applied at 37°C for 60 minutes at a dilution of 1:20 for our normal tissue TMA and 1:300 for cancer tissues. For the purpose of validating and comparing antibodies, the normal tissue TMA and a subset of 2214 cancer tissues (666 breast cancers and 1191 non-breast cancers, including 622 non-breast adenocarcinomas) were also analyzed by the rabbit recombinant monoclonal TRPS1 antibody EPR16171 (#ab209664, Abcam) at a dilution of 1:2000 and by the rabbit polyclonal TRPS1 antibody PA5-84874 (Invitrogen/ThermoFisher) at a dilution of 1:100 with an otherwise identical protocol. Bound antibody was then visualized using the EnVision Kit (Agilent; #K5007) according to the manufacturer's directions. The sections were counterstained with haemalaun. For tumor tissues, the percentage of positive neoplastic cells was estimated, and the staining intensity was semiquantitatively recorded (0, 1+, 2+, and 3+). For statistical analyses, the staining results were categorized into 4 groups. Tumors without any staining were considered negative. Tumors with 1+ staining intensity in $\leq 70\%$ of tumor cells and 2+ intensity in $\leq 30\%$ of tumor cells were considered weakly positive. Tumors with 1+ staining intensity in $> 70\%$ of tumor cells, 2+ intensity in 31% to 70%, or 3+ intensity in $\leq 30\%$ were considered moderately positive. Tumors with 2+ intensity in $> 70\%$ or 3+ intensity in $> 30\%$ of tumor cells were considered strongly positive. This scoring system has been used in numerous TMA studies and led to the identification of numerous known and novel prognostic molecular features in various tumor types.[29–32]

Statistics

Statistical calculations were performed with JMP 17 software (SAS Institute Inc.). Contingency tables and the χ^2 test were performed to search for associations between TRPS1 immunostaining and tumor phenotype. Survival curves were calculated according to Kaplan-Meier. The log-rank test was applied to detect significant differences between groups. Sensitivity and specificity for the detection of breast cancers were calculated according to the following formulas: sensitivity = number of true positives divided by the number of true positives plus number of false negatives; specificity = number of true negatives divided by the number of true negatives plus number of false positives.

RESULTS

Technical Issues

A total of 16,818 (87.6%) of 19,201 tumor samples were interpretable in our TMA analysis. Noninterpretable samples demonstrated either a lack of unequivocal tumor cells or loss of the tissue spots during technical procedures. At least 4 samples of each normal tissue type were evaluated.

TRPS1 in Normal Tissues

TRPS1 staining was always nuclear and strongest in luminal epithelial cells of breast glands. A moderate to strong nuclear staining was also observed in epithelial cells of the endometrium (stroma cells were also positive, but weaker), glial cells of the brain, sebaceous glands, and suprabasal cells of non-keratinizing squamous epithelium. A weak to moderate TRPS1 positivity was observed in various other epithelial (salivary glands, gallbladder, fallopian tube, respiratory epithelium, renal tubuli, amnion, thyroid gland, and parathyroid gland), muscular (smooth muscle and myometrium), neuronal (ganglion cells in the intestine), and germinal (spermatogonia) cell types. In other organs, TRPS1 staining of epithelial cells was only occasionally detected, such as, for example, in atrophic acinar cells of the prostate. A nuclear TRPS1 staining of variable intensity was also regularly seen in stromal cells of various tissues, especially in the case of tissue reparation (probably fibroblasts). Representative images are shown in Figure 1. A similar nuclear staining was observed in all these cell types when the anti-TRPS1 antibody EPR16171 was used (Supplemental Fig. 1, Supplemental Digital Content 1, http://links.lww.com/PAS/B785). As compared with MSVA-512R, EPR16171 resulted in additional cytoplasmic staining in basal cells of several non-keratizing squamous epithelia (Supplemental Fig. 1, Supplemental Digital Content 1, http://links.lww.com/PAS/B785), whereas PA-84874 showed significant additional cytoplasmic staining in many different normal tissues, including, for example, smooth muscle cells, kinocilia of the epididymis, parietal cells of the stomach, and lymphocytes, as well as nuclear staining in Leydig cells of the testis (Supplemental Fig. 2, Supplemental Digital Content 2, http://links.lww.com/PAS/B786). The additional stainings observed with EPR16171 and PA-84874 were considered antibody-specific cross-reactivities.

TRPS1 in Tumor Tissues

TRPS1 immunostaining was detectable in 2482 (14.8%) of the 16,818 analyzable tumors, including 614 (3.7%) with weak, 800 (4.8%) with moderate, and 1068 (6.4%) with strong immunostaining. Overall, 86 (56.6%) of 152 tumor categories showed TRPS1 positivity with 36 (23.7%) tumor categories including at least one case with strong staining (Table 1). The highest rate of TRPS1 positivity was found in various types of breast cancers (51.4% to 100%), soft tissue tumors (up to 100%), salivary gland tumors (up to 46.2%), squamous cell carcinomas of various sites of origin (up to 34.7%), and in diverse gynecological cancers (up to 40.0%). TRPS1 positivity only occurred in 1.8% of 1083 urothelial neoplasms. Representative images are shown in Figure 2. A ranking order of TRPS1-positive and strongly positive tumors is given in Figure 3. The relationship between TRPS1 expression and clinically important histopathological and molecular tumor features in breast cancer, ovarian cancer, endometrial carcinoma, and squamous cell carcinomas from different sites is shown in Table 2. In invasive breast cancer of no special type, low TRPS1 expression was linked to high grade ($P = 0.0547$), advanced pathologic tumor stage ($P < 0.0001$), nodal metastasis ($P = 0.0571$), loss of estrogen receptor expression ($P < 0.0001$), loss of progesterone receptor expression ($P < 0.0001$), and triple-negative status ($P < 0.0001$) but was unrelated to overall patient survival ($P = 0.8016$; Supplemental Fig 4, Supplemental Digital Content 3, http://links.lww.com/PAS/B787). A combined analysis of 677 squamous cell carcinomas from 11 different sites did not reveal associations between TRPS1 expression and tumor phenotype. TRPS1 expression was also unrelated to human papillomavirus infection status ($P = 0.1159$; Supplemental Table 1, Supplemental Digital Content 4, http://links.lww.com/PAS/B788). In our prostate cancers, the rate of TRPS1 positivity increased from Gleason grade 3+3 (0%) to Gleason grade 5+5 (3.8%) and recurrent adenocarcinomas under therapy (7.6%; $P = 0.0164$). TRPS1 immunostaining was unrelated to the histopathological tumor phenotype in ovarian and endometrial cancer.

TRPS1 Versus GATA3 Expression

A comparative description of TRPS1 versus GATA3 expression is given in Figure 4 and in Supplemental Table 2 (Supplemental Digital Content 5, http://links.lww.com/PAS/B789) for 11,891 tumors with data on both markers. The data show that positivity for both markers is frequent (47.4% to 100%) in breast cancer, and also occurs in a fraction of salivary gland tumors (up to 30.0%) while it is exceedingly rare in all other cancer entities. Of 1159 tumors positive for TRPS1 and GATA3, 97.5% were derived from either the breast or salivary glands. Considering that TRPS1 should stain positive in breast and salivary gland tumors while GATA3 should label breast, urothelial, and salivary gland tumors the numbers of positive cases outside of these tumor categories were assessed. TRPS1 positivity occurred in 4.7% of 9835 tumors from 55 of 114 "additional categories." GATA3 staining was seen in 2.3% of 8980

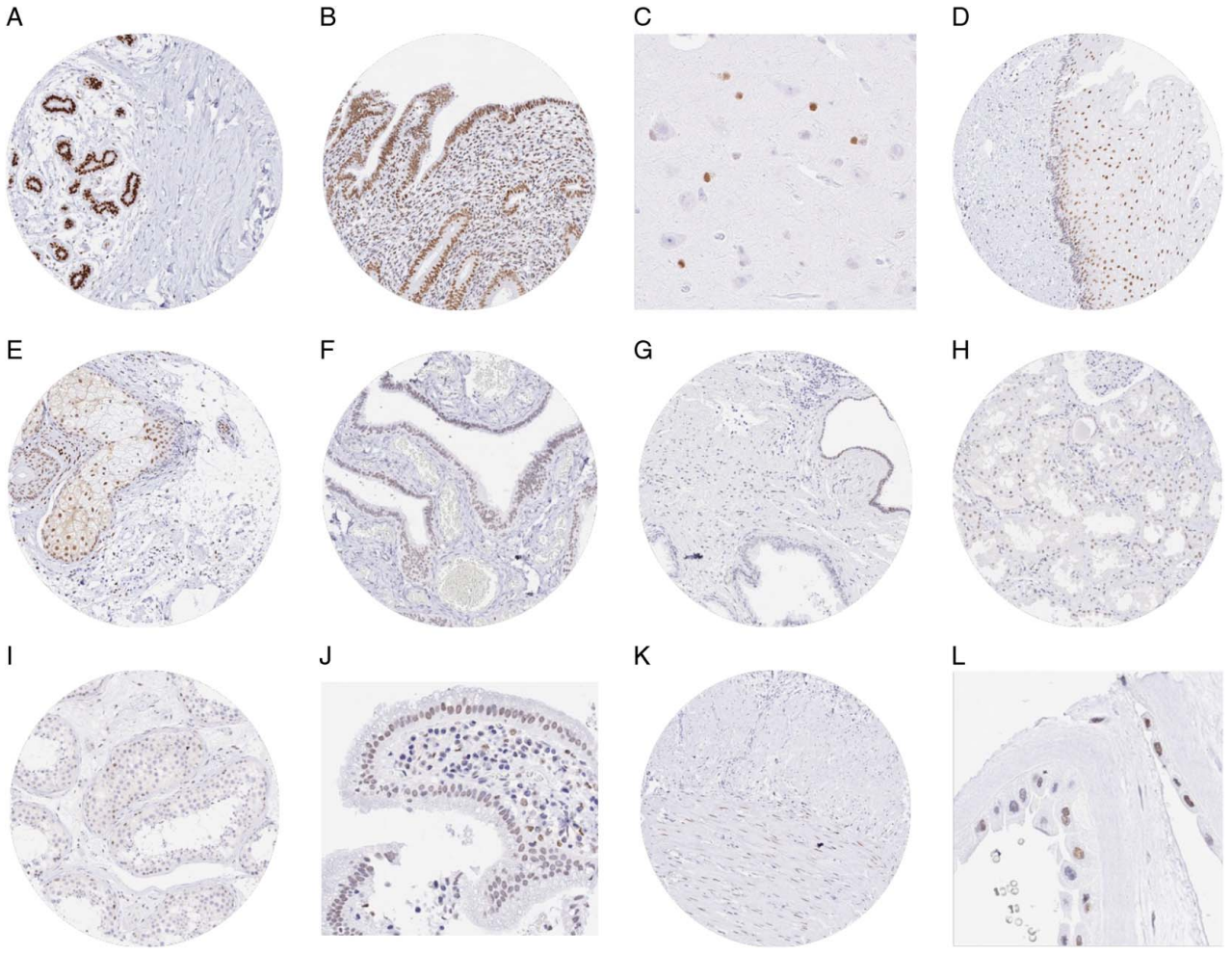

FIGURE 1. TRPS1 immunostaining of normal tissues. The panels show a strong nuclear staining of breast epithelial cells (A) epithelial cells of the endometrium (B), and glial cells in the brain (C). Variable nuclear staining also occurred in suprabasal cells of esophageal squamous epithelium (D), sebaceous glands of the skin (E), epithelial cells of the fallopian tube (F), atrophic epithelial cells of the prostate (G), some tubular cells of the kidney (H), spermatogonia of the testis (I), epithelial cells of the gallbladder (J), smooth muscle cells of the appendix (K), and amnion cells (L).

tumors from 50 of 109 "additional categories." Positivity for both TRPS1 and GATA3 occurred in 47.4% to 100% of breast cancers, and up to 30% of salivary gland tumors, but in only 29 (0.3%) of 9835 tumors from 134 other cancer entities.

Comparison of Sensitivity and Specificity by Using Different TRPS1 Assays

Staining results for the antibodies MSVA-512R, EPR16171, and PA-84874 on 2214 cancers and the respective sensitivities and specificities for the detection of breast cancers and triple-negative breast cancers are given in Table 3. Examples of tumors stained with the 3 antibodies are given in Supplemental Figure 3 (Supplemental Digital Content 6, http://links.lww.com/PAS/B790). At the selected conditions, sensitivity for the detection of breast cancer/triple-negative breast cancer was higher for EPR16171 (85%/86%) and PA5-84874 (85%/86%) as for MSVA-512R (83%/76%). Specificity for the distinction between breast cancers and non-breast adenocarcinomas/non-breast cancers was highest for MSVA-512R (92%/89%), followed by PA5-84874 (67%/47%) and EPR16171 (58%/44%).

DISCUSSION

Considering conflicting previous results on TRPS1 IHC in cancer and the large scale of our study, emphasis was placed on a thorough validation of our assay. The International Working Group for Antibody Validation has proposed that an acceptable antibody validation for IHC on formalin-fixed tissues must include either a comparison of the findings obtained by 2 different independent antibodies or a comparison with expression data obtained by another independent method.[33] Both methods were applied in this project. The rather ubiquitous expression of *TRPS1* RNA in normal tissues which was suggested by compiled RNA data (https://www.proteinatlas.org) from 3

TABLE 1. TRPS1 Immunostaining in Human Tumors

Tumor entity		On TMA (n)	TRPS1 immunostaining result				
			Analyzable (n)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)
Tumors of the skin	Pilomatricoma	35	21	61.9	23.8	4.8	9.5
	Basal cell carcinoma of the skin	89	54	96.3	1.9	1.9	0.0
	Benign nevus	29	26	100.0	0.0	0.0	0.0
	Squamous cell carcinoma of the skin	145	127	84.3	14.2	0.0	1.6
	Malignant melanoma	65	59	98.3	1.7	0.0	0.0
	Malignant melanoma Lymph node metastasis	86	72	100.0	0.0	0.0	0.0
	Merkel cell carcinoma	48	28	100.0	0.0	0.0	0.0
Tumors of the head and neck	Squamous cell carcinoma of the larynx	109	93	90.3	9.7	0.0	0.0
	Squamous cell carcinoma of the pharynx	60	49	65.3	30.6	4.1	0.0
	Oral squamous cell carcinoma (floor of the mouth)	130	107	75.7	22.4	0.9	0.9
	Pleomorphic adenoma of the parotid gland	50	47	40.4	14.9	38.3	6.4
	Warthin tumor of the parotid gland	104	98	100.0	0.0	0.0	0.0
	Adenocarcinoma, NOS (papillary cystadenocarcinoma)	14	10	70.0	30.0	0.0	0.0
	Salivary duct carcinoma	15	12	75.0	16.7	8.3	0.0
	Acinic cell carcinoma of the salivary gland	181	146	97.9	0.7	0.7	0.7
	Adenocarcinoma NOS of the salivary gland	109	87	89.7	3.4	3.4	3.4
	Adenoid cystic carcinoma of the salivary gland	180	106	59.4	18.9	18.9	2.8
	Basal cell adenocarcinoma of the salivary gland	25	21	81.0	14.3	0.0	4.8
	Basal cell adenoma of the salivary gland	101	86	87.2	10.5	2.3	0.0
	Epithelial-myoepithelial carcinoma of the salivary gland	53	51	58.8	9.8	23.5	7.8
	Mucoepidermoid carcinoma of the salivary gland	343	295	92.9	3.7	3.1	0.3
	Myoepithelial carcinoma of the salivary gland	21	18	61.1	16.7	16.7	5.6
	Myoepithelioma of the salivary gland	11	9	66.7	0.0	33.3	0.0
	Oncocytic carcinoma of the salivary gland	12	11	90.9	0.0	9.1	0.0
	Polymorphous adenocarcinoma, low grade, of the salivary gland	41	26	53.8	19.2	19.2	7.7
	Pleomorphic adenoma of the salivary gland	53	36	80.6	11.1	8.3	0.0
Tumors of the lung, pleura and thymus	Adenocarcinoma of the lung	196	189	97.4	2.1	0.0	0.5
	Squamous cell carcinoma of the lung	80	74	90.5	8.1	1.4	0.0
	Small cell carcinoma of the lung	16	11	100.0	0.0	0.0	0.0
	Mesothelioma, epithelioid	40	27	92.6	7.4	0.0	0.0
	Mesothelioma, biphasic	77	48	91.7	4.2	4.2	0.0
	Thymoma	29	28	96.4	3.6	0.0	0.0
	Lung (NET)	29	28	96.4	0.0	3.6	0.0
Tumors of the female genital tract	Squamous cell carcinoma of the vagina	78	61	90.2	3.3	3.3	3.3
	Squamous cell carcinoma of the vulva	157	140	87.1	12.9	0.0	0.0
	Squamous cell carcinoma of the cervix	136	124	96.8	1.6	0.8	0.8
	Adenocarcinoma of the cervix	23	19	100.0	0.0	0.0	0.0
	Endometrioid endometrial carcinoma	338	269	85.5	9.7	3.3	1.5
	Endometrial serous carcinoma	86	60	83.3	15.0	1.7	0.0
	Carcinosarcoma of the uterus	57	47	76.6	17.0	6.4	0.0
	Endometrial carcinoma, high grade, G3	13	10	60.0	30.0	0.0	10.0
	Endometrial clear cell carcinoma	9	8	100.0	0.0	0.0	0.0
	Endometrioid carcinoma of the ovary	130	119	76.5	9.2	12.6	1.7
	Serous carcinoma of the ovary	580	537	76.2	14.5	8.4	0.9
	Mucinous carcinoma of the ovary	101	88	96.6	1.1	2.3	0.0
	Clear cell carcinoma of the ovary	51	48	93.8	2.1	4.2	0.0
	Carcinosarcoma of the ovary	47	46	71.7	21.7	6.5	0.0
	Granulosa cell tumor of the ovary	44	37	100.0	0.0	0.0	0.0
	Leydig cell tumor of the ovary	4	4	100.0	0.0	0.0	0.0
	Sertoli cell tumor of the ovary	1	1	100.0	0.0	0.0	0.0

TABLE 1. (*continued*)

Tumor entity		On TMA (n)	TRPS1 immunostaining result				
			Analyzable (n)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)
	Sertoli Leydig cell tumor of the ovary	3	3	100.0	0.0	0.0	0.0
	Steroid cell tumor of the ovary	3	3	100.0	0.0	0.0	0.0
	Brenner tumor	41	38	100.0	0.0	0.0	0.0
Tumors of the breast	Invasive breast carcinoma of no special type	1764	1533	11.1	7.1	29.5	52.3
	Lobular carcinoma of the breast	363	304	8.9	6.6	30.9	53.6
	Medullary carcinoma of the breast	34	27	14.8	25.9	33.3	25.9
	Tubular carcinoma of the breast	29	18	0.0	5.6	44.4	50.0
	Mucinous carcinoma of the breast	65	38	10.5	13.2	39.5	36.8
	Phyllodes tumor of the breast	50	35	48.6	8.6	25.7	17.1
Tumors of the digestive system	Adenomatous polyp, low-grade dysplasia	50	50	100.0	0.0	0.0	0.0
	Adenomatous polyp, high-grade dysplasia	50	50	100.0	0.0	0.0	0.0
	Adenocarcinoma of the colon	2483	2247	99.9	0.1	0.0	0.0
	Gastric adenocarcinoma, diffuse type	215	198	99.5	0.0	0.5	0.0
	Gastric adenocarcinoma, intestinal type	215	202	98.0	1.5	0.5	0.0
	Gastric adenocarcinoma, mixed type	62	62	100.0	0.0	0.0	0.0
	Adenocarcinoma of the esophagus	83	70	98.6	0.0	0.0	1.4
	Squamous cell carcinoma of the esophagus	76	61	91.8	8.2	0.0	0.0
	Squamous cell carcinoma of the anal canal	91	75	70.7	26.7	1.3	1.3
	Cholangiocarcinoma	58	57	98.2	1.8	0.0	0.0
	Gallbladder adenocarcinoma	51	48	91.7	2.1	6.3	0.0
	Gallbladder Klatskin tumor	42	36	97.2	2.8	0.0	0.0
	Hepatocellular carcinoma	312	274	99.3	0.7	0.0	0.0
	Ductal adenocarcinoma of the pancreas	659	638	98.6	1.1	0.2	0.2
	Pancreatic/ampullary adenocarcinoma	98	97	100.0	0.0	0.0	0.0
	Acinar cell carcinoma of the pancreas	18	18	100.0	0.0	0.0	0.0
	GIST	62	58	77.6	13.8	1.7	6.9
	Appendix (NET)	25	20	100.0	0.0	0.0	0.0
	Colorectal (NET)	12	11	100.0	0.0	0.0	0.0
	Ileum (NET)	53	52	100.0	0.0	0.0	0.0
	Pancreas (NET)	101	97	100.0	0.0	0.0	0.0
	Colorectal (NEC)	14	12	91.7	8.3	0.0	0.0
	Ileum (NEC)	8	8	100.0	0.0	0.0	0.0
	Gallbladder (NEC)	4	4	100.0	0.0	0.0	0.0
	Pancreas (NEC)	14	14	100.0	0.0	0.0	0.0
Tumors of the urinary system	Noninvasive papillary urothelial carcinoma, pTa G2 low grade	177	174	100.0	0.0	0.0	0.0
	Noninvasive papillary urothelial carcinoma, pTa G2 high grade	141	140	100.0	0.0	0.0	0.0
	Noninvasive papillary urothelial carcinoma, pTa G3	219	138	99.3	0.7	0.0	0.0
	Urothelial carcinoma, pT2-4 G3	735	631	97.1	1.7	0.5	0.6
	Squamous cell carcinoma of the bladder	22	20	95.0	5.0	0.0	0.0
	Small cell NEC of the bladder	23	16	100.0	0.0	0.0	0.0
	Sarcomatoid urothelial carcinoma	25	16	87.5	12.5	0.0	0.0
	Urothelial carcinoma of the kidney pelvis	62	52	100.0	0.0	0.0	0.0
	Clear cell renal cell carcinoma	1286	1131	100.0	0.0	0.0	0.0
	Papillary renal cell carcinoma	368	316	100.0	0.0	0.0	0.0
	Clear cell (tubulo) papillary renal cell carcinoma	26	23	100.0	0.0	0.0	0.0
	Chromophobe renal cell carcinoma	170	151	100.0	0.0	0.0	0.0
	Oncocytoma of the kidney	257	222	100.0	0.0	0.0	0.0
Tumors of the male genital organs	Adenocarcinoma of the prostate, Gleason 3+3	83	78	100.0	0.0	0.0	0.0
	Adenocarcinoma of the prostate, Gleason 4+4	80	69	100.0	0.0	0.0	0.0
	Adenocarcinoma of the prostate, Gleason 5+5	85	79	96.2	3.8	0.0	0.0

	Adenocarcinoma of the prostate (recurrence)	258	237	92.4	5.1	1.7	0.8
	Small cell NEC of the prostate	19	7	100.0	0.0	0.0	0.0
	Seminoma	682	665	100.0	0.0	0.0	0.0
	Embryonal carcinoma of the testis	54	48	100.0	0.0	0.0	0.0
	Leydig cell tumor of the testis	31	23	100.0	0.0	0.0	0.0
	Sertoli cell tumor of the testis	2	2	50.0	0.0	50.0	0.0
	Sex cord-stromal tumor of the testis	1	1	100.0	0.0	0.0	0.0
	Spermatocytic tumor of the testis	1	1	100.0	0.0	0.0	0.0
	Yolk sac tumor	53	46	100.0	0.0	0.0	0.0
	Teratoma	53	49	100.0	0.0	0.0	0.0
	Squamous cell carcinoma of the penis	92	67	86.6	10.4	3.0	0.0
Tumors of endocrine organs	Adenoma of the thyroid gland	113	110	100.0	0.0	0.0	0.0
	Papillary thyroid carcinoma	391	353	99.7	0.3	0.0	0.0
	Follicular thyroid carcinoma	154	145	100.0	0.0	0.0	0.0
	Medullary thyroid carcinoma	111	105	100.0	0.0	0.0	0.0
	Parathyroid gland adenoma	43	27	100.0	0.0	0.0	0.0
	Anaplastic thyroid carcinoma	45	42	90.5	7.1	2.4	0.0
	Adrenal cortical adenoma	50	46	100.0	0.0	0.0	0.0
	Adrenal cortical carcinoma	28	28	100.0	0.0	0.0	0.0
	Pheochromocytoma	50	50	100.0	0.0	0.0	0.0
Tumors of hematopoietic and lymphoid tissues	Hodgkin's lymphoma	103	93	100.0	0.0	0.0	0.0
	B-SLL/B-CLL	50	39	100.0	0.0	0.0	0.0
	DLBCL	113	93	97.8	2.2	0.0	0.0
	Follicular lymphoma	88	71	100.0	0.0	0.0	0.0
	T-cell non-Hodgkin's lymphoma	25	21	90.5	4.8	4.8	0.0
	Mantle cell lymphoma	18	12	100.0	0.0	0.0	0.0
	Marginal zone lymphoma	16	12	100.0	0.0	0.0	0.0
	DLBCL in the testis	16	15	100.0	0.0	0.0	0.0
	Burkitt lymphoma	5	1	100.0	0.0	0.0	0.0
Tumors of soft tissue and bone	Tendosynovial giant cell tumor	45	29	44.8	37.9	17.2	0.0
	Granular cell tumor	53	28	100.0	0.0	0.0	0.0
	Leiomyoma	50	50	100.0	0.0	0.0	0.0
	Leiomyosarcoma	94	88	93.2	5.7	1.1	0.0
	Liposarcoma	145	123	95.9	2.4	0.8	0.8
	MPNST	15	14	71.4	7.1	21.4	0.0
	Myofibrosarcoma	26	25	96.0	4.0	0.0	0.0
	Angiosarcoma	74	55	98.2	0.0	1.8	0.0
	Angiomyolipoma	91	88	100.0	0.0	0.0	0.0
	Dermatofibrosarcoma protuberans	21	17	100.0	0.0	0.0	0.0
	Ganglioneuroma	14	14	100.0	0.0	0.0	0.0
	Kaposi sarcoma	8	3	100.0	0.0	0.0	0.0
	Neurofibroma	117	117	98.3	1.7	0.0	0.0
	Sarcoma (NOS)	74	68	83.8	11.8	0.0	4.4
	Paraganglioma	41	41	100.0	0.0	0.0	0.0
	Ewing sarcoma	23	15	93.3	0.0	0.0	6.7
	Rhabdomyosarcoma	7	6	83.3	16.7	0.0	0.0
	Schwannoma	122	121	100.0	0.0	0.0	0.0
	Synovial sarcoma	12	10	0.0	20.0	50.0	30.0
	Osteosarcoma	44	25	52.0	16.0	8.0	24.0
	Chondrosarcoma	40	18	88.9	11.1	0.0	0.0
	Rhabdoid tumor	5	5	80.0	20.0	0.0	0.0
	Solitary fibrous tumor	17	17	94.1	0.0	5.9	0.0

B-CLL indicates chronic lymphocytic leukemia, B-cell type; B-SLL, small lymphocytic lymphoma, B-cell type; DLBCL, diffuse large B-cell lymphoma; GIST, gastrointestinal stromal tumor; MPNST, malignant peripheral nerve sheath tumor; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; NOS, not otherwise specified.

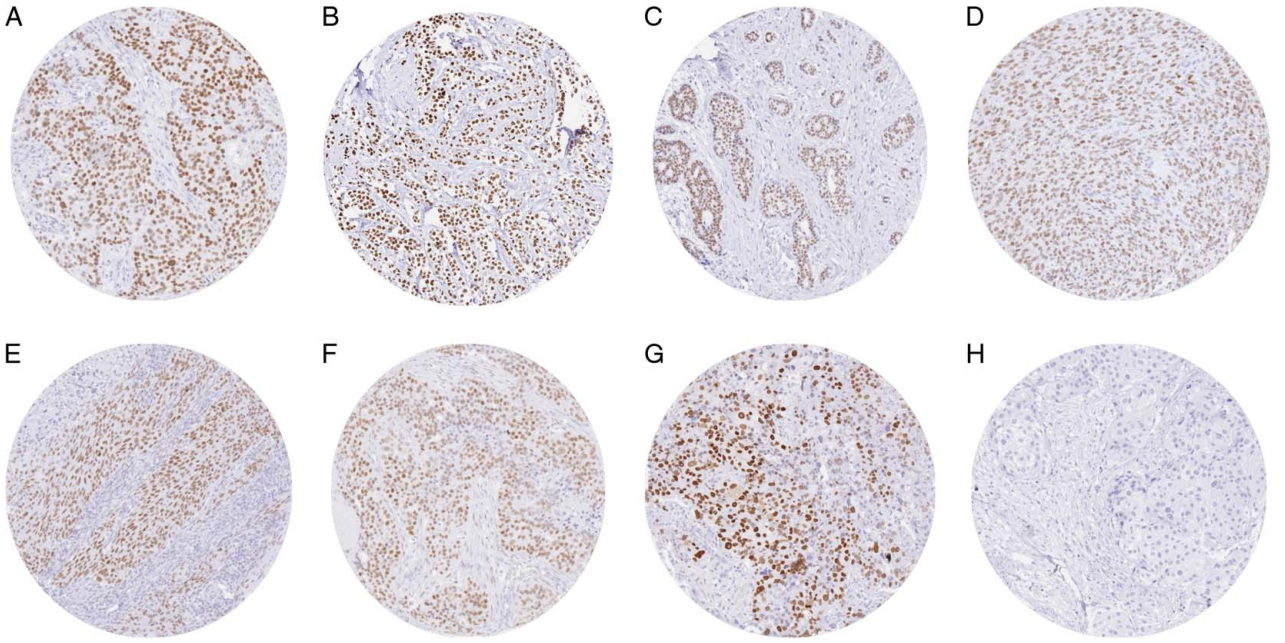

FIGURE 2. TRPS1 immunostaining in cancer. The panels show a nuclear TRPS1 positivity of variable intensity in an invasive breast carcinoma of no special type (A) a lobular breast carcinoma (B), an adenoid cystic carcinoma of the parotid gland (C), a synovial sarcoma (D), a squamous cell carcinoma of oral cavity (E), a high-grade serous carcinoma of the ovary (F), and a recurrent adenocarcinoma of the prostate (G). TRPS1 staining is lacking in urothelial carcinoma of the urinary bladder (H).

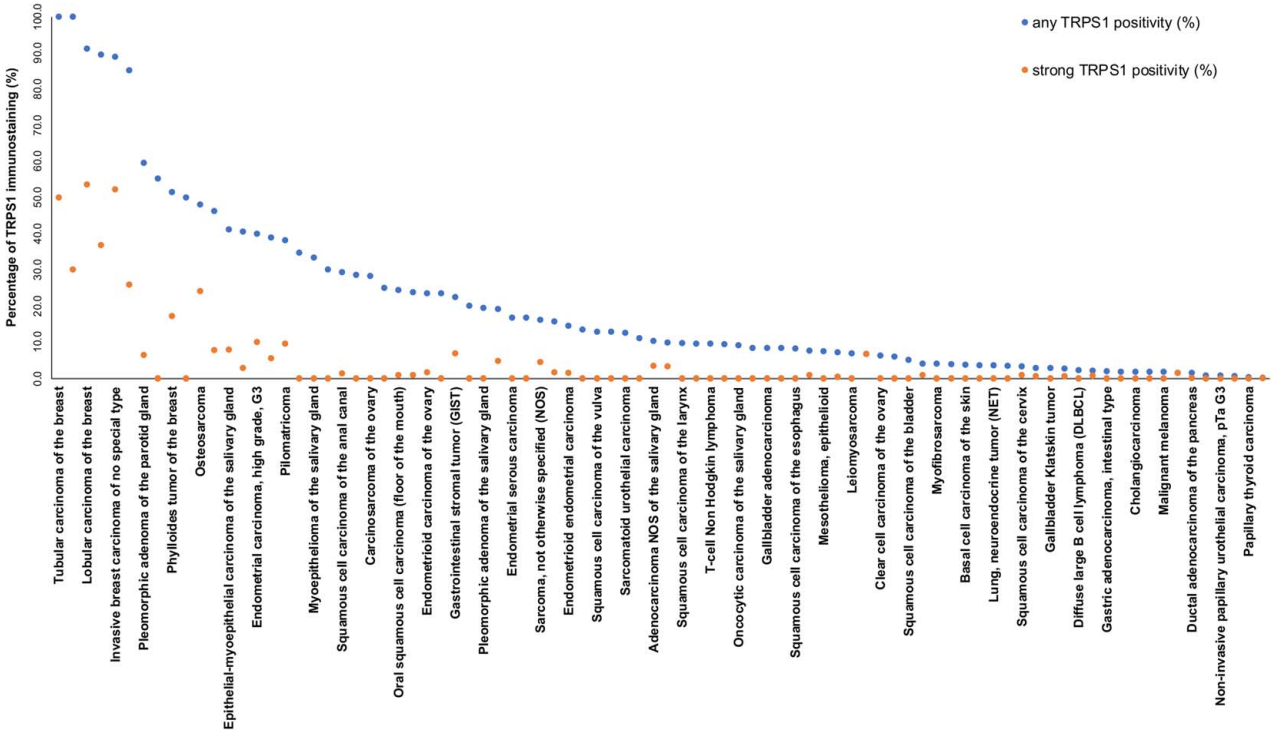

FIGURE 3. Ranking order of TRPS1 immunostaining in tumors. Both the percentage of positive cases (blue dots) and the percentage of strongly positive cases (orange dots) are shown.

TABLE 2. TRPS1 and Tumor Phenotype

		TRPS1 immunostaining result				
	n	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	*P*
Invasive breast carcinoma of no special type						
pT1	697	6.5	5.3	30.0	58.2	< 0.0001
pT2	600	13.2	8.7	29.0	49.2	—
pT3-4	120	18.3	6.7	31.7	43.3	—
G1	166	4.8	6.0	36.1	53.0	0.0547
G2	758	10.3	7.0	27.8	54.9	—
G3	533	12.0	7.1	30.8	50.1	—
pN0	635	9.0	7.1	30.7	53.2	0.0571
pN1	316	11.4	6.3	31.6	50.6	—
pN2	112	14.3	11.6	30.4	43.8	—
pN3	68	20.6	11.8	22.1	45.6	—
pM0	177	5.1	6.2	37.3	51.4	0.0072
pM1	111	15.3	9.9	37.8	36.9	—
HER2 negative	832	7.8	6.7	29.1	56.4	0.5888
HER2-positive	114	7.9	7.0	35.1	50.0	—
ER-negative	197	18.3	8.6	39.1	34.0	< 0.0001
ER-positive	706	4.7	6.2	27.2	61.9	—
PR-negative	374	14.2	9.1	34.5	42.2	< 0.0001
PR-positive	564	3.4	5.0	26.8	64.9	—
Non-triple negative	742	5.0	6.5	28.8	59.7	< 0.0001
Triple negative	133	22.6	9.0	33.1	35.3	—
Endometrioid endometrial carcinoma						
pT1	79	83.5	11.4	2.5	2.5	0.9265
pT2	22	81.8	9.1	4.5	4.5	—
pT3-pT4	23	82.6	13.0	4.3	0.0	—
pN0	37	75.7	16.2	5.4	2.7	0.1875
pN+	21	85.7	4.8	0.0	9.5	—
Endometrioid carcinoma of the ovary						
pT1	25	68.0	12.0	20.0	0.0	0.2860
pT2	5	80.0	0.0	20.0	0.0	—
pT3	6	100.0	0.0	0.0	0.0	—
pN0	22	68.2	13.6	18.2	0.0	0.0609
pN1	9	100.0	0.0	0.0	0.0	—
Serous carcinoma of the ovary						
pT1	33	81.8	6.1	9.1	3.0	0.0650
pT2	45	71.1	8.9	17.8	2.2	—
pT3	266	80.8	13.5	5.3	0.4	—
pN0	84	77.4	10.7	10.7	1.2	0.8677
pN1	169	78.7	12.4	7.7	1.2	—
Squamous cell carcinomas of different sites*						
pT1	225	87.1	10.7	1.8	0.4	0.5931
pT2	226	85.8	12.8	0.9	0.4	—
pT3	117	82.9	16.2	0.9	0.0	—
pT4	109	89.0	10.1	0.0	0.9	—
pN0	266	86.8	11.7	0.8	0.8	0.2684
pN+	260	84.2	14.6	1.2	0.0	—
G1	26	84.6	11.5	3.8	0.0	0.2907
G2	370	87.8	11.1	0.3	0.8	—
G3	234	83.3	14.5	1.7	0.4	—

*Oral, pharynx, larynx, esophagues, cervix, vagina, vulva, penis, anal, skin, and lung.

ER indicates estrogen receptor; G, grade; pM, pathologic status of distant metastasis; pN, pathologic lymph node status; PR, progesterone receptor; pT, pathologic tumor stage.

public databases[34–36] could largely be confirmed if the MSVA-512R antibody was highly concentrated (1:20). Given the expression in virtually all organs and cell type specificity of TRPS1 expression, a comparison with a method based on disaggregated tissue is suboptimal for TRPS1 antibody validation, however. Pivotal evidence for the validity of our assay thus comes from a confirmation of all TRPS1-positive cell types seen by MSVA-512R by the independent antibodies EPR16171 and PA5-84874. The additional cytoplasmic staining of basal cells in squamous epithelia that were only seen by EPR16171 and the additional cytoplasmic staining of several cell types by PA5-84874 were considered antibody-specific cross-reactivities. It is of note that the use of a very broad selection of normal tissue categories (n = 76) for antibody validation maximizes the probability of detecting cross-reactivities because virtually all proteins occurring in normal human adult cells are included in the validation process.

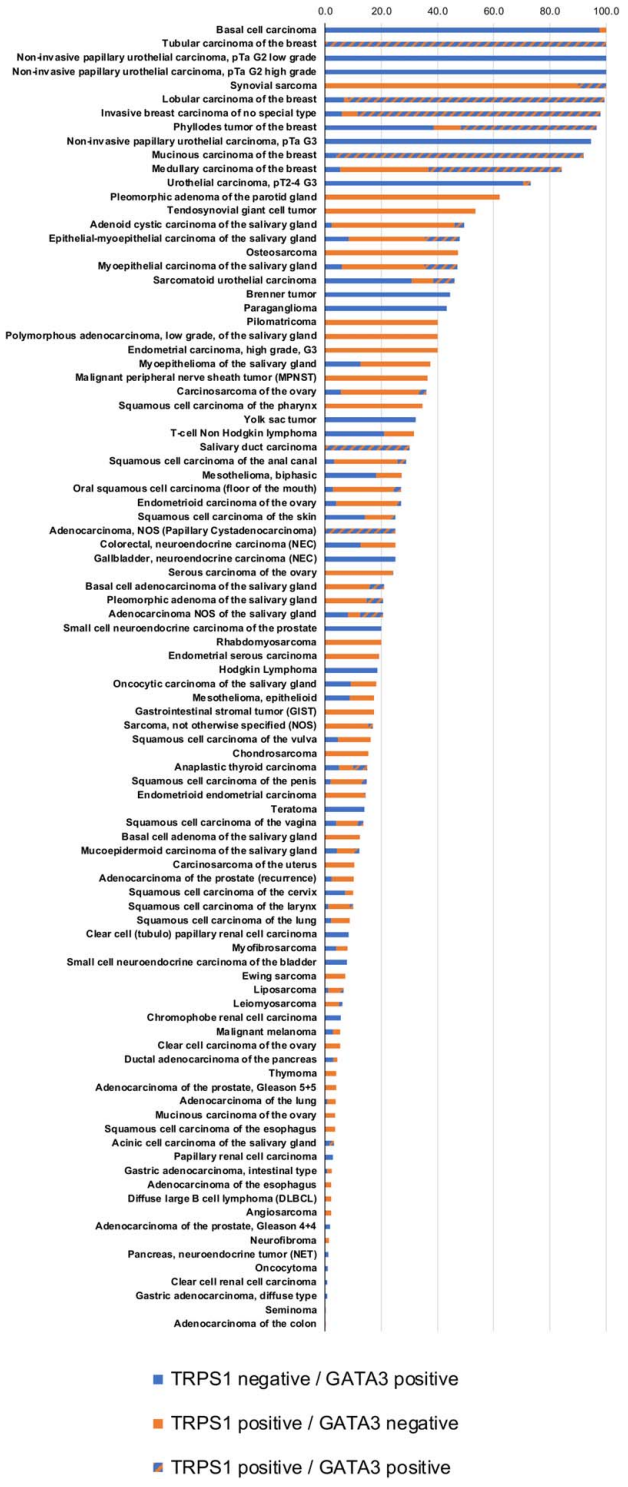

FIGURE 4. TRPS1 and GATA3 immunostaining in tumors.

Our initial tumor screening revealed that TRPS1 expression levels were highest in breast cancer but other tumors were also TRPS1-positive. For this study, our staining protocol was thus adjusted to the highest possible dilution that still resulted in a strong TRPS1 staining of a large fraction of breast cancers but as low as possible staining of other tumors and normal tissues. Under these conditions, our analysis of 19,201 tumors from 152 different tumor categories demonstrated a strong predominance of TRPS1 staining in breast cancers. This was expected based on RNA expression data from different tumor types which are described in The Cancer Genome Atlas database (https://www.cancer.gov/tcga) and data from previous IHC studies.[10,15] Of tumors, 94% with strong and 74% with moderate TRPS1 positivity were breast neoplasms in this study. Our TRPS1 positivity rate of 75% across all breast cancer subtypes is in the middle range of the 41% to 92% TRPS1 positivity rates that have previously been described in studies employing IHC on cohorts of 50 to 1061 breast cancers.[7,26,37] The comparison of our data with two different TRPS1 assays suggests that a higher TRPS1 positivity rate in breast cancer goes along with a marked decrease in specificity for the detection of both breast cancer and triple-negative breast cancer.

Several studies have suggested that TRPS1 IHC could complement GATA3 in its role as a breast cancer marker in case of tumor masses of uncertain derivation.[10,11,26,27] The significantly higher positivity rate for TRPS1 (77%) than for GATA3 (55%) in our triple-negative breast cancers is consistent with data from several earlier reports and underscores the diagnostic utility of TRPS1 in these tumors. Other studies have reported 79% to 91% TRPS1 positivity in triple-negative breast cancer.[10,26,38,39] These figures are again higher than the 43% to 56% positivity rates described for GATA3 in these tumors.[10,26,27]

A comprehensive overview of TRPS1 expression across human tumors represents the key result of our study. TRPS1 immunostaining in various further tumor entities, often at a lower level than in breast cancer, parallels the expression of TRPS1 in a broad range of different normal cell types. Proteins expressed in normal cell types are mostly retained after malignant transformation.[40] The highest rates of TRPS1 positivity among non-breast tumors were seen in subtypes of soft tissue tumors, salivary gland tumors, squamous cell carcinomas, and gynecological cancers. This is in line with individual reports describing TRPS1 positivity in these entities (Fig. 5). Several authors recently described frequent TRPS1 positivity in salivary gland tumors. In line with our data, these authors described the highest TRPS1 positivity rates in tumors with ductal differentiation, such as pleomorphic adenomas, adenoid cystic carcinomas, or epithelial-myoepithelial carcinomas while TRPS1 expression was low in acinus cell carcinomas or mucoepidermoid carcinomas.[41,42] Based on a TRPS1 positivity in 13 of 14 squamous cell carcinomas but in only 1 of 10 basal cell carcinomas of the skin, Liu et al[19] suggested that the distinction of these tumors may represent another application for TRPS1 IHC. Although our assay was designed to have low sensitivity in non-breast cancers, the 16% positivity in our squamous cell carcinomas as compared with 4% in basal cell carcinomas of the skin is consistent with this notion.

TRPS1 expression in non-breast gynecological tumors—as seen in our study—has also been described by

TABLE 3. Sensitivity and Specificity of Anti-TRPS1 Antibodies

			TRPS1 MSVA-512R					TRPS1 EPR16171					TRPS1 PA5-84874				
	Tumor entity	Category for sensitivity/ specificity calculation	n	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	n	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	n	Negative (%)	Weak (%)	Moderate (%)	Strong (%)
Tumors of the skin	Basal cell carcinoma	Non-breast cancer	14	100.0	0.0	0.0	0.0	26	38.5	57.7	3.8	0.0	25	52.0	36.0	12.0	0.0
	Squamous cell carcinoma of the skin	Non-breast cancer	30	83.3	16.7	0.0	0.0	37	24.3	45.9	27.0	2.7	36	25.0	38.9	33.3	2.8
Tumors of the head and neck	Squamous cell carcinoma of the larynx	Non-breast cancer	50	86.0	14.0	0.0	0.0	53	22.6	34.0	34.0	9.4	55	16.4	47.3	34.5	1.8
	Squamous cell carcinoma of the pharynx	Non-breast cancer	49	65.3	30.6	4.1	0.0	55	25.5	32.7	32.7	9.1	57	24.6	33.3	35.1	7.0
	Oral squamous cell carcinoma (floor of the mouth)	Non-breast cancer	60	65.0	33.3	0.0	1.7	70	14.3	31.4	47.1	7.1	73	12.3	35.6	49.3	2.7
Tumors of the female genital tract	Squamous cell carcinoma of the vagina	Non-breast cancer	28	89.3	7.1	3.6	0.0	28	46.4	35.7	7.1	10.7	28	53.6	32.1	3.6	10.7
	Squamous cell carcinoma of the vulva	Non-breast cancer	74	89.2	10.8	0.0	0.0	74	20.3	37.8	31.1	10.8	75	16.0	44.0	25.3	14.7
	Squamous cell carcinoma of the cervix	Non-breast cancer	77	98.7	0.0	0.0	1.3	78	66.7	16.7	14.1	2.6	76	56.6	27.6	13.2	2.6
	Carcinosarcoma of the uterus	Non-breast adenocarcinoma	19	68.4	26.3	5.3	0.0	15	33.3	26.7	40.0	0.0	14	28.6	28.6	14.3	28.6
	Endometrioid carcinoma of the ovary	Non-breast adenocarcinoma	32	65.6	12.5	18.8	3.1	21	28.6	28.6	19.0	23.8	21	23.8	23.8	23.8	28.6
	Serous carcinoma of the ovary	Non-breast adenocarcinoma	79	58.2	25.3	13.9	2.5	71	25.4	26.8	21.1	26.8	70	18.6	30.0	15.7	35.7
	Mucinous carcinoma of the ovary	Non-breast adenocarcinoma	24	95.8	4.2	0.0	0.0	16	75.0	0.0	18.8	6.3	15	80.0	0.0	20.0	0.0
	Clear cell carcinoma of the ovary	Non-breast adenocarcinoma	21	85.7	4.8	9.5	0.0	16	25.0	31.3	31.3	12.5	16	43.8	25.0	18.8	12.5
	Carcinosarcoma of the ovary	Non-breast adenocarcinoma	17	76.5	17.6	5.9	0.0	12	41.7	25.0	16.7	16.7	10	30.0	40.0	10.0	20.0
Tumors of the breast	Invasive breast carcinoma of no special type	Breast cancer	462	19.7	7.8	26.6	45.9	422	5.5	5.5	9.0	80.1	430	6.0	8.4	5.3	80.2
	Lobular carcinoma of the breast	Breast cancer	142	10.6	6.3	31.0	52.1	118	2.5	4.2	9.3	83.9	119	3.4	4.2	4.2	88.2
	Medullary carcinoma of the breast	Breast cancer	8	0.0	25.0	25.0	50.0	7	0.0	0.0	14.3	85.7	7	0.0	0.0	14.3	85.7
	Tubular carcinoma of the breast	Breast cancer	2	0.0	0.0	50.0	50.0	2	0.0	0.0	0.0	100.0	2	0.0	0.0	0.0	100.0
	Mucinous carcinoma of the breast	Breast cancer	6	0.0	0.0	33.3	66.7	6	0.0	0.0	0.0	100.0	7	0.0	0.0	0.0	100.0
	Hormone receptor-positive breast cancer *	—	425	16.9	7.1	28.0	48.0	402	3.0	3.5	8.2	85.3	410	3.7	5.9	4.9	85.6
	Triple-negative breast cancer *	Triple-negative breast cancer	58	32.8	17.2	22.4	27.6	58	13.8	15.5	10.3	60.3	59	13.6	20.3	5.1	61.0

TABLE 3. (*continued*)

			TRPS1 MSVA-512R					TRPS1 EPR16171					TRPS1 PA5-84874				
	Tumor entity	**Category for sensitivity/ specificity calculation**	**n**	**Negative (%)**	**Weak (%)**	**Moderate (%)**	**Strong (%)**	**n**	**Negative (%)**	**Weak (%)**	**Moderate (%)**	**Strong (%)**	**n**	**Negative (%)**	**Weak (%)**	**Moderate (%)**	**Strong (%)**
Tumors of the digestive system	Adenocarcinoma of the colon	Non-breast adenocarcinoma	79	100.0	0.0	0.0	0.0	69	94.2	5.8	0.0	0.0	70	94.3	5.7	0.0	0.0
	Gastric adenocarcinoma, diffuse type	Non-breast adenocarcinoma	67	100.0	0.0	0.0	0.0	40	75.0	15.0	10.0	0.0	43	86.0	4.7	2.3	7.0
	Gastric adenocarcinoma, intestinal type	Non-breast adenocarcinoma	69	95.7	2.9	1.4	0.0	42	54.8	33.3	2.4	9.5	42	52.4	35.7	4.8	7.1
	Adenocarcinoma of the esophagus	Non-breast adenocarcinoma	70	98.6	0.0	0.0	1.4	43	58.1	23.3	16.3	2.3	45	57.8	24.4	8.9	8.9
	Squamous cell carcinoma of the esophagus	Non-breast cancer	61	91.8	8.2	0.0	0.0	39	15.4	46.2	23.1	15.4	35	25.7	40.0	22.9	11.4
	Squamous cell carcinoma of the anal canal	Non-breast cancer	30	80.0	20.0	0.0	0.0	37	5.4	54.1	35.1	5.4	38	5.3	34.2	44.7	15.8
	Ductal adenocarcinoma of the pancreas	Non-breast adenocarcinoma	80	98.8	1.3	0.0	0.0	64	79.7	14.1	6.3	0.0	68	82.4	14.7	2.9	0.0
	Pancreatic/ampullary adenocarcinoma	Non-breast adenocarcinoma	28	100.0	0.0	0.0	0.0	23	78.3	17.4	4.3	0.0	23	87.0	13.0	0.0	0.0
	Acinar cell carcinoma of the pancreas	Non-breast adenocarcinoma	5	100.0	0.0	0.0	0.0	4	75.0	25.0	0.0	0.0	4	75.0	0.0	25.0	0.0
Tumors of the male genital organs	Adenocarcinoma of the prostate, Gleason 3+3	Non-breast adenocarcinoma	78	100.0	0.0	0.0	0.0	64	84.4	10.9	4.7	0.0	65	96.9	3.1	0.0	0.0
	Adenocarcinoma of the prostate, Gleason 4+4	Non-breast adenocarcinoma	69	100.0	0.0	0.0	0.0	55	45.5	27.3	27.3	0.0	57	71.9	19.3	8.8	0.0
	Adenocarcinoma of the prostate, Gleason 5+5	Non-breast adenocarcinoma	79	96.2	3.8	0.0	0.0	67	20.9	52.2	17.9	9.0	62	62.9	22.6	8.1	6.5
	Squamous cell carcinoma of the penis	Non-breast cancer	55	87.3	9.1	3.6	0.0	72	30.6	23.6	30.6	15.3	71	15.5	39.4	36.6	8.5
Breast cancers vs non-breast adenocarcinomas				Sensitivity		0.83			Sensitivity		0.95			Sensitivity		0.95	
				Specificity		0.92			Specificity		0.58			Specificity		0.67	
Breast cancers vs non-breast adenocarcinomas/non-breast cancers				Sensitivity		0.83			Sensitivity		0.95			Sensitivity		0.95	
				Specificity		0.89			Specificity		0.44			Specificity		0.47	
Triple-negative breast cancers vs non-breast adenocarcinomas				Sensitivity		0.76			Sensitivity		0.86			Sensitivity		0.86	
				Specificity		0.92			Specificity		0.58			Specificity		0.67	
Triple-negative breast cancers vs non-breast adenocarcinomas/ non-breast cancers				Sensitivity		0.76			Sensitivity		0.86			Sensitivity		0.86	
				Specificity		0.89			Specificity		0.44			Specificity		0.47	

*Included in the other subtypes of breast cancers.

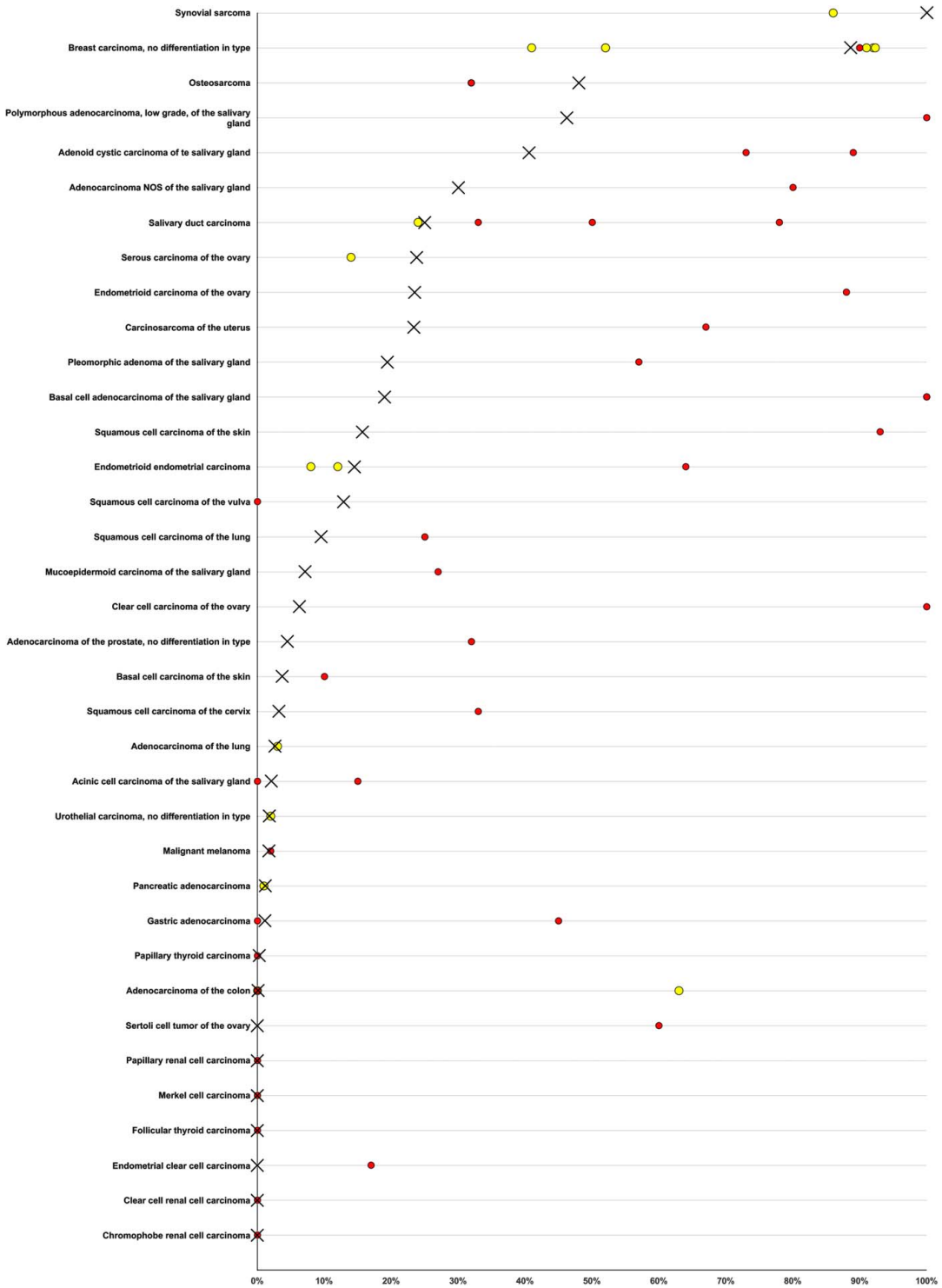

FIGURE 5. Comparison with previous TRPS1 literature. An "X" indicates the fraction of TRPS1-positive tumors in the present study, and dots indicate the reported frequencies from the literature for comparison; red dots mark studies with <100 analyzed tumors and yellow dots mark studies with ≥ 100 analyzed tumors.

other authors.[10,15] Skvarca et al[39,43] found TRPS1 staining in 8% of 184 endometrioid G3 tumors of the endometrium and identified a significant association between high TRPS1 expression and unfavorable disease outcome. Rammal et al[39,43] reported TRPS1 positivity from 71% of 69 endometrial endometrioid adenocarcinomas, 1 out of 5 endometrioid carcinomas, and 17% of 250 ovarian tumors. Chen and colleagues have shown TRPS1 staining in

20% of 152 ovarian and 12% of 152 endometrial carcinomas, and Ai and colleagues found 8% to 14% TRPS1 staining in 251 ovarian carcinomas of different subtypes. Our TRPS1 positivity in 100% of our synovial sarcomas is consistent with a recent report by Cloutier et al[20] showing 86% TRPS1-positive cases among 165 synovial sarcomas. Based on published RNA expression data and their ChIP-seq (chromatin immunoprecipitation DNA sequencing) findings these authors suggested that TRPS1 overexpression may be mediated by the enrichment of several transcriptionally activating histone modifications in these tumors. TRPS1 positivity in 29% of our 14 malignant peripheral nerve sheath tumors while there was a complete lack of TRPS1 staining in 121 Schwannomas could potentially indicate a role of TRPS1 in the malignant transformation of nerve sheath tumors and suggest that detectable TRPS1 staining could represent a feature of malignancy in these tumors. Earlier studies also reported a high sensitivity and specificity of TRPS1 for primary extramammary Paget disease,[44] and differential staining of TRPS1 in various types of malignant and benign cutaneous sweat glands.[45]

Several studies have proposed oncogenic[46,47] or tumor suppressive[5,6] roles for TRPS1. The availability of clinic-pathologic data for several tumor cohorts enabled us to interrogate the potential clinical significance of aberrant TRPS1 expression in vivo. That a reduced TRPS1 expression could be observed in invasive breast carcinomas with no special type with unfavorable histopathological tumor features is in line with the more aggressive behavior of TRPS1-negative cancers. Other studies evaluating TRPS1 in 152 and 341 breast carcinomas had earlier reported a significant link between low TRPS1 expression and poor prognosis[15,25] or unfavorable tumor features.[25] Two further studies on 152 and 180 cancers have failed to find evidence for a clinical role of TRPS1 expression in breast cancer, however.[15,48] The link between low rates of TRPS1 expression and unfavorable tumor features in cancers derived from TRPS1 expressing normal tissues is consistent with either a tumor suppressive role of TRPS1 or a progressive loss of TRPS1 expression during cellular dedifferentiation that is unrelated to cell functions required for tumor progression. It is well known that neoplastic cells continuously lose the expression of nonessential proteins during cancer progression.[49,50] In line with a possible oncogenic role of TRPS1, a relationship between high TRPS1 expression and unfavorable histologic tumor features was recorded for prostate cancer in this study. This is consistent with recent data by Bachert et al[12] describing high TRPS1 expression in metastatic prostate cancers.

Our data confirm the recently proposed utility of the combination TRPS1 and GATA3 IHC for the assessment of tumor masses of unknown origin[10,26,27] and for the distinction of breast cancer from other specific tumor entities.[51–53] A major advantage of this combination lies in the virtual absence of TRPS1 positivity in urothelial carcinomas, the tumor entity with the second highest GATA3 positivity rate after breast cancer. In our analysis, a combined GATA3 and TRPS1 positivity was almost exclusively seen in neoplasms of the breast and the salivary glands. Only 2.5% of 1159 GATA3/TRPS1 dual-positive tumors were of non-breast and non-salivary gland origin in this study. That only 1.4% of 842 urothelial neoplasms were TRPS1-positive underscores the utility of TRPS1 for the distinction of breast cancer from urothelial carcinoma. That "TRPS1 alone" positivity was more common than "GATA3 alone" positivity again reflects the common low-level expression of TRPS1 in many different normal cell types.

CONCLUSION

The results of our study demonstrate that TRPS1 is expressed in a broad range of normal cell types with the highest expression levels in normal breast epithelial cells. Because the average TRPS1 expression in cancers is highest in breast neoplasms, immunostaining protocols can be defined that result in high sensitivity and considerable specificity of TRPS1 staining for breast (or salivary gland) cancer although various other tumor entities can show—a usually less intense—TRPS1 positivity.

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