



Trophoblast cell surface antigen 2 (TROP2) expression in human tumors: A tissue microarray study on 18,563 tumors.

David Dum¹, Anne Menz¹, Doris Höflmayer¹, Maximilian Lennartz¹, Niclas C. Blessin¹, Christoph Fraune¹, Christian Bernreuther¹, Guido Sauter¹, Ria Uhlig¹, Waldemar Wilczak¹, Stefan Steurer¹, Sarah Minner¹, Ronald Simon¹, Eike Burandt¹, Till Krech¹



¹Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction and Objectives

Trophoblast cell surface antigen 2 (TROP2) is the target of sacituzumab govitecan (SG), an antibody-drug conjugate that was recently approved for previously treated triple negative breast cancer and urothelial carcinomas. In order to learn more about the role of TROP2 for tumor biology and identify other tumor types that might benefit from anti-TROP2 therapies, a comprehensive analysis of TROP2 protein expression across virtually all types of human normal and neoplastic tissues was performed.

Materials & Methods

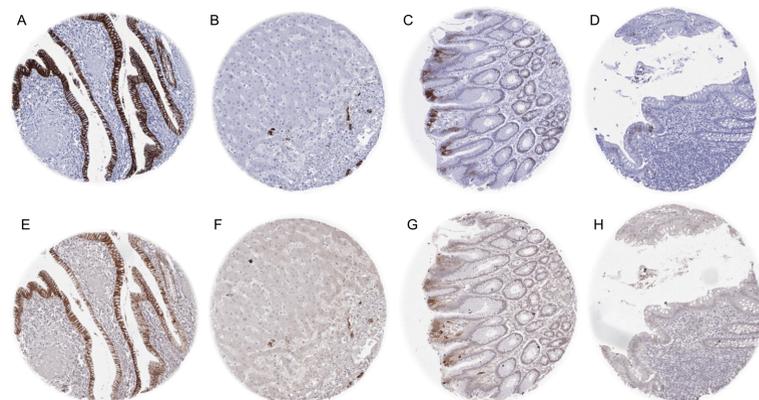
Tissue microarrays containing 18,563 samples from 150 different tumor types and subtypes as well as 608 samples of 76 different normal tissue types was analyzed by immunohistochemistry.

Immunostaining protocol and controls



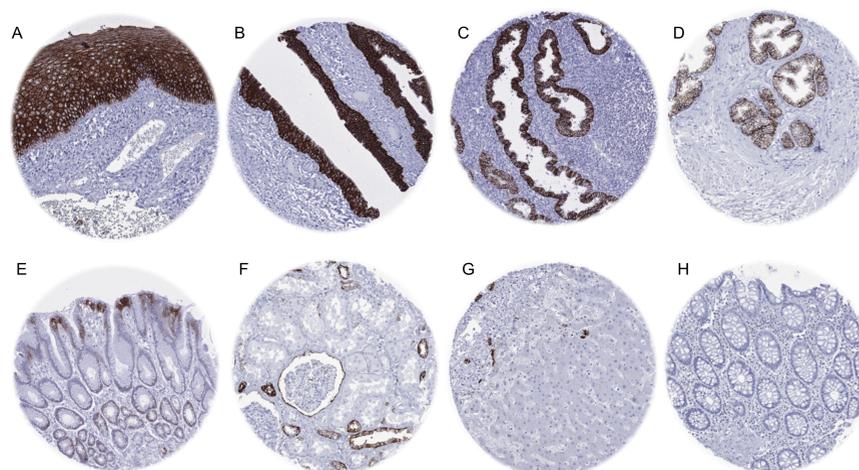
- Antibody: MS validated antibodies, clone MSVA-733R, Recombinant Rabbit IgG, Dilution: 1:150
- Antigen retrieval: 5 min at 121°C (autoclave) in pH 7.8 buffer
- Controls:
 - Positive: Strong membranous Trop-2 immunostaining should be seen in bile ducts of the liver.
 - Negative: Liver hepatocytes should not show any Trop-2 immunostaining.

Antibody validation by comparison of antibodies. The panels show a complete concordance of staining results obtained by two independent TROP2 antibodies. Using MSVA-733R, the stainings show a strong predominantly membranous staining of gallbladder epithelium (A) and of intrahepatic bile ducts (B) while staining is less intense and focused on surface epithelial cells and glands (weaker) of the stomach (C) and limited to few interspersed epithelial cells in the colon (D). Using clone AF650, nearly identical stainings are seen in gallbladder (E), liver (F), stomach (G), and the colon (H). The images A-D and E-H were taken from consecutive tissue sections. Due to the polyclonal nature of AF650, background staining is slightly higher than seen for MSVA-733R.



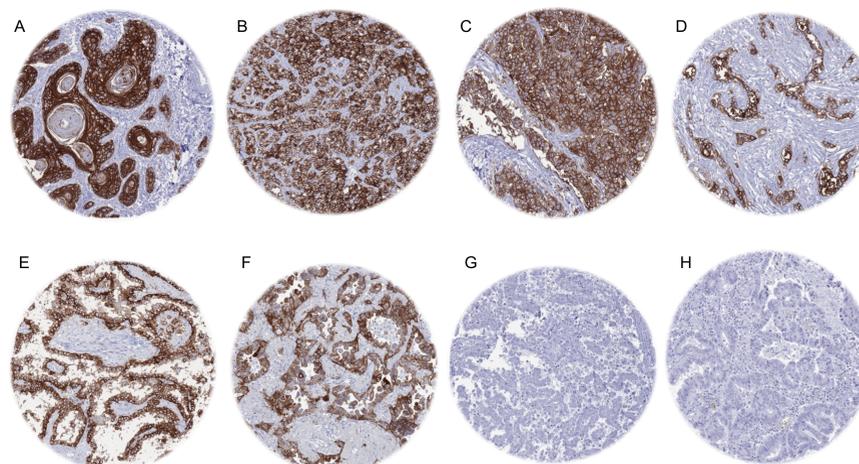
TROP2 immunostaining examples

TROP2 in normal tissues



TROP2 immunostaining was always membranous and found in many epithelial cell types. The panel shows strong TROP2 positivity of surface epithelial cells of the tonsil (A), urothelium of the urinary bladder (B), and the endometrium (C) as well as in acinar and basal cells of the prostate (D). TROP2 staining is somewhat weaker and largely limited to the most apical elements of the surface epithelium in the stomach antrum (E), distal tubuli and the visceral layer of the Bowman capsule of the kidney (F), and intrahepatic bile ducts of the liver (G). TROP2 immunostaining is lacking in colon epithelial cells (H).

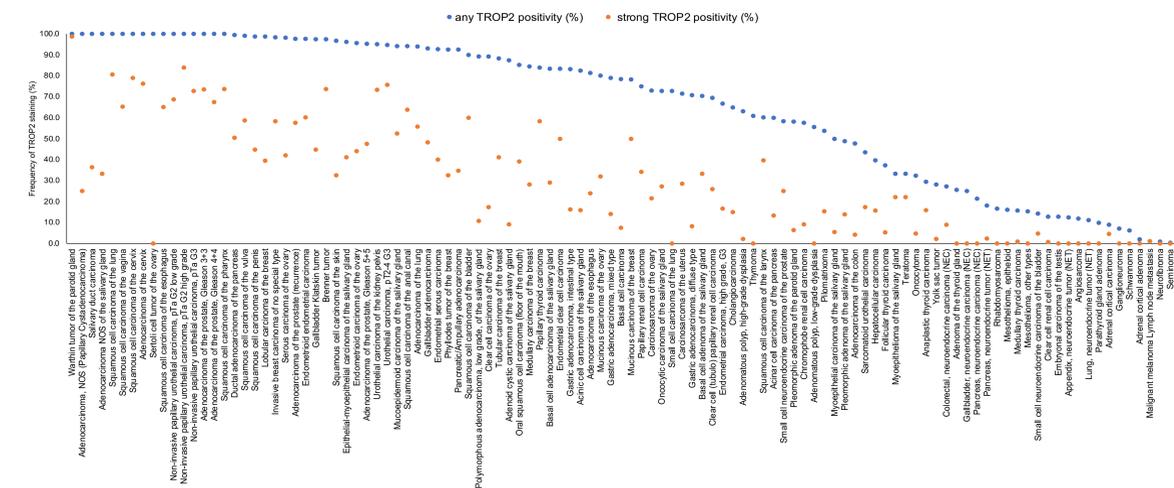
TROP2 in tumor tissues



Strong, membranous and cytoplasmatic TROP2 immunostaining in a squamous cell carcinoma of the oral cavity (A), a recurrent adenocarcinoma (Gleason 5+5=10) of the prostate (B), a breast cancer of no special type (C), a gastric adenocarcinoma (D), a papillary carcinoma of the thyroid (E), and an adenocarcinoma of the lung (F). TROP2 staining is absent in an epitheloid pleural mesothelioma (G) and a colorectal adenocarcinoma (H).

Ranking and prognostic value of TROP2 in tumor tissues

Ranking order of TROP2 immuno-staining in cancers. Both the frequency of positive cases (blue dots) and the frequency of strongly positive cases (orange dots) are shown.



High TROP2 expression was linked to adverse tumor features in colorectal cancer, gastric adenocarcinoma and papillary thyroid cancer. Low TROP2 expression was linked to advanced stage in urothelial carcinoma, high stage and grade and “triple negative receptor status” in breast cancer, as well as with high stage and grade in papillary renal cell carcinomas. No associations were found in clear cell renal cell, ovarian, pancreatic, and endometrium carcinomas.

		TROP2 immunostaining result					P	
		n	neg. (%)	weak (%)	mod. (%)	strong (%)		
Primary Tumor	pT1	79	54.4	35.4	8.9	1.3	0.0069	
	pT2	406	53.2	34.5	7.4	4.9		
	pT3	1157	53.5	32.2	11.2	3.1		
	pT4	419	49.6	30.1	13.4	6.9		
	pN0	1088	57.9	30.6	8.1	3.4	<0.0001	
	pN+	963	46.6	34.2	14.1	5.1		
	Venous Invasion	V1	1479	54.9	31.3	9.6	4.2	0.0012
	V2	543	45.9	35.5	14.2	4.4		
	Lymphatic Invasion	L0	661	58.9	31.2	7	3	<0.0001
	L1	1368	49.3	33	12.9	4.8		
Tumor localization	left colon	1122	52	35	8.9	4.1	0.0273	
	right colon	425	53.2	29.2	13.4	4.2		
	defective	86	51.2	34.9	10.5	3.5	0.9848	
	proficient	1071	51.1	35.5	9.4	4		
RAS mutation status	mutated	325	48.3	38.5	9.8	3.4	0.2722	
	wildtype	414	54.8	32.6	8.5	4.1		
BRAF mutation status	mutated	14	42.9	14.3	28.6	14.3	0.1262	
	wildtype	90	56.7	28.9	10	4.4		
Primary Tumor	pT1	151	11.9	17.9	9.3	60.9	0.0487	
	pT2	76	26.3	14.5	11.8	47.4		
	pT3-4	96	12.5	11.5	7.3	68.8		
	pN0	89	20.2	13.5	7.9	58.4	0.0013	
Regional Lymph Nodes	pN+	122	4.1	10.7	7.4	77.9		
	pN2	148	1.4	6.1	35.8	56.8		
Primary Tumor	pT1	899	0.9	6.8	30.1	62.2	0.0024	
	pT2	796	2	8.3	33.5	56.2		
	pT3-4	182	4.9	8.8	35.2	51.1		
Grade	G1	215	0.5	2.8	22.3	74.4	<0.0001	
	G2	1050	1.8	5.6	33.2	59.3		
	G3	659	2.3	12.3	33.2	52.2		
Regional Lymph Nodes	pN0	872	1.9	7.1	32.3	58.6	0.286	
	pN1	406	1.5	8.6	31.3	58.6		
	pN2	148	1.4	6.1	35.8	56.8		
Primary Tumor	pN3	100	4	6	44	46		
	negative	995	1.9	9.9	30.3	57.9	0.6044	
	positive	125	0.8	12	32.8	54.4		
ER status	negative	233	17	19.7	24.9	53.6	<0.0001	
	positive	822	1.9	8	31.4	58.6		
	PR status	negative	457	2.2	13.1	29.5	55.1	0.0305
Primary Tumor	positive	654	1.7	7.8	31.5	59		
	no	858	2	9	31.2	57.8	0.001	
	yes	158	1.3	20.3	24.1	54.4		

		TROP2 immunostaining result					P
		n	neg. (%)	weak (%)	mod. (%)	strong (%)	
Primary Tumor	pTa G2 low	125	0	1.6	29.6	68.8	<0.0001
	pTa G2 high	106	0	1.9	14.2	84	
	pTa G3	133	0.8	6	26.3	66.9	
Regional Lymph Nodes	pT2	122	3.3	0.8	13.9	82	
	pT3	205	3	3.4	17.2	76.4	
	pT4	97	8.2	2.1	16.5	73.2	
	pN+	170	3.2	3.2	16	77.6	0.9068
Laurén type	diffuse	66	30.3	24.2	43.9	1.5	0.0208
	intestinal	81	22.2	38.3	29.6	9.9	
	mixed	57	21.1	22.8	42.1	14	
Primary Tumor	pT1-2	48	35.4	27.1	29.2	8.3	0.1352
	pT3	114	22.8	21.9	42.1	13.2	
	pT4	111	17.1	27.9	46.8	8.1	
Regional Lymph Nodes	pN0	69	31.9	30.4	30.4	7.2	0.0246
	pN1	58	27.6	19	39.7	13.8	
	pN2	55	16.4	18.2	47.3	18.2	
Mismatch repair status	pN3	90	16.7	30	47.8	5.6	
	MMR defective	32	46.9	18.8	18.8	15.6	0.0002
	MMR proficient	233	14.6	24.9	48.9	11.6	
ISUP stage	1	41	17.1	22	19.5	41.5	0.0005
	2	134	14.9	17.2	25.4	42.5	
	3	81	39.5	14.8	28.4	17.3	
	4	7	57.1	14.3	14.3	14.3	
Fuhrmann grade	1	4	0	50	0	50	<0.0001
	2	183	14.2	17.5	25.1	43.2	
	3	83	41	18.1	24.1	16.9	
Thoenes grade	4	11	45.5	9.1	36.4	9.1	
	1	58	13.8	17.2	22.4	46.6	0.1706
	2	157	26.1	17.8	24.2	31.8	
UICC stage	3	18	33.3	16.7	33.3	16.7	
	1	102	23.5	17.6	22.5	36.3	0.0097
	2	15	13.3	13.3	46.7	26.7	
Primary Tumor	3	5	90	0	0	20	
	4	11	36.4	0	54.5	9.1	
	1	208	21.2	17.8	24.5	36.5	0.0009
Regional Lymph Nodes	2	48	10.4	18.8	33.3	37.5	
	3-4	33	54.5	9.1	21.2	15.2	
	≥1	25	36	4	28	32	0.4776
Distant Metastasis	≥1	15	33.3	6.7	46.7	13.3	
	0	27	25.9	11.1	25.9	37	0.2267
	≥1	12	41.7	8.3	41.7	8.3	