Journal of Histochemistry & Cytochemistry 59(7) 701–710
© The Author(s) 2011
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1369/0022155411410430
http://jhc.sagepub.com
SAGE

Expression of Trop2 Cell Surface Glycoprotein in Normal and Tumor Tissues: Potential Implications as a Cancer Therapeutic Target

Lara P. Stepan, Esther S. Trueblood, Kari Hale, John Babcook, Luis Borges, and Claire L. Sutherland

Departments of Oncology (LPS,LB,CLS), Pathology (EST), and Molecular Sciences (KH), Amgen Inc., Seattle, Washington, and Department of Protein Sciences (JB), Amgen Inc., Burnaby, British Columbia, Canada.

Summary

Trop2 is a cell-surface glycoprotein reported to be overexpressed in various types of adenocarcinomas with minimal expression in normal tissues. Recent findings that Trop2 expression correlates with tumor aggressiveness have increased interest in Trop2 as a potential target for cancer immunotherapy. The goal of this study was to extensively evaluate Trop2 expression at the transcript and protein levels in normal and tumor tissues. It was determined that Trop2 is overexpressed on some carcinomas relative to the corresponding normal tissue. However, in human and mouse, Trop2 is highly expressed at both the transcript and protein levels on several essential normal tissues. The findings suggest that the development of therapeutic agents to target Trop2 may require strategies that target Trop2 on malignant tissues in order to minimize potential toxicities to essential normal tissues that also express high levels of Trop2. (J Histochem Cytochem 59:701–710, 2011)

Keywords

Trop2, tissue distribution, human, murine, cancer

Trop2 is a type-I transmembrane glycoprotein (Alberti et al. 1992; Fornaro et al. 1995), originally identified by its high expression on human trophoblast cells (Lipinski et al. 1981). Trop2 may transmit an intracellular signal as engagement with antibodies causes calcium flux (Ripani et al. 1998) and Trop2 expression can activate ERK (Cubas et al. 2010). A ligand for Trop2 has not been identified, and its natural function is not well understood. Loss of function mutations in Trop2 underlie gelatinous drop-like corneal dystrophy (GDLD) (Tsujikawa et al. 1999), a rare autosomal recessive disorder that is characterized by amyloid formation in the cornea leading to blindness (Weber and Bable 1980). Recent findings suggest that at least in the cornea, Trop2 is required for proper subcellular localization of tight junction proteins and maintenance of epithelial barrier function (Nakatsukasa et al. 2010).

Trop2 is a paralog of epithelial-specific cell adhesion molecule (EpCAM)/Trop1, a growth stimulatory molecule (Munz et al. 2009; Zanna et al. 2007) that is a therapeutic target for epithelial cancers in several ongoing antibody-based clinical studies (Sebastian et al. 2009). Trop2 is overexpressed by various types of human carcinomas including

Received for publication December 20, 2010; accepted April 1, 2011.

Corresponding Author:
Claire L. Sutherland, Department of Oncology, Amgen Inc., 1201 Amgen Court West, Seattle, WA 98119
Email: sutherlc@amgen.com

Supplementary material for this article is available on the *Journal of Histochemistry & Cytochemistry* Web site at http://jhc.sagepub.com/supplemental.

ovarian (Bignotti et al. 2010), colorectal (Ohmachi et al. 2006), pancreatic (Fong, Moser, et al. 2008), gastric (Muhlmann et al. 2009), and squamous cell carcinoma of the oral cavity (Fong, Spizzo, et al. 2008) compared with the corresponding normal tissue. High Trop2 expression appears to correlate with poor patient prognosis in these studies, leading to the suggestion that Trop2 could be a therapeutic target for various carcinomas.

The potential role of Trop2 expression in tumor cells is under investigation. Targeting Trop2 with antibodies or RNA interference inhibits colony formation by colon cancer cell lines in vitro and tumorigenesis in mice (Wang et al. 2008), suggesting that Trop2 may regulate cell growth. Conversely, ectopic expression of Trop2 promotes anchorage-independent growth in vitro (Wang et al. 2008) and tumorigenesis in mice (Cubas et al. 2010). Trop2 may also have oncogenic activity through a bicistronic *CYCLIN D1-TROP2* mRNA frequently expressed by ovarian, colonic, and endometrial tumors (Guerra et al. 2008). Mutagenesis studies indicate that both the *TROP2* and *CYCLIN D1* moieties in the chimera contribute to cell transformation (Guerra et al. 2008).

To further evaluate Trop2 as a potential target for antibody-based tumor therapy, we analyzed Trop2 mRNA and protein expression levels across human and murine tissues and compared Trop2 expression in normal and tumor tissues. In contrast to literature reports, we used a much broader panel of tumors and normal tissues and compared levels of expression across all tissues. Most literature reports focused just on the comparison of malignant tissues versus the normal counterparts. Our findings suggest that Trop2 is widely expressed in healthy tissues and that therapeutic methods to specifically target Trop2 on tumor tissues may be needed to avoid potential toxicity to Trop2-expressing normal tissues.

Materials and Methods

Quantitative RT-PCR (qRT-PCR) Analysis of Trop2 mRNA Expression

Commercially available RNAs from human, murine, and tumor tissues were purchased from Ambion Inc. (Austin, TX) and Stratagene Inc. (La Jolla, CA). Each of these samples, representing pools of RNAs from 10 or more individuals, were processed in parallel to RNA extracted in-house from OCT-embedded, individual patient tissues. Tumor tissues from individual patients were verified by hematoxylin and eosin staining to contain >60% tumor tissue. The OCT-embedded tissues were homogenized by TisssueLyzer (Qiagen, Valencia, CA) and RNA was extracted using the *mir*Vana total RNA extraction kit (Ambion Inc.). All RNAs were quality checked by A260 reading (NanoDrop, Wilmington, DE) and Bioanalyzer tRNA profile (Agilent Technologies, Santa Clara, CA). RNAs were treated with RNase-*free* DNase (Ambion Inc.) and reverse transcribed using random hexamers in the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA).

qRT-PCR was performed on cDNA samples using primers to the housekeeping gene, human or murine *ACTB*, and to the target gene, human or murine *TROP2*. The human ACTB primers were 5′-CCT GGC ACC CAG CAC AA-3′ and 5′-GCC GAT CCA CAC GGA GTA CT-3′ with probe 6FAM-ATC AAG ATC ATT GCT CCT CCT GAG CG- TAMRA. The murine ACTB primers were 5′-TCC TTC GTT GCC GGT CCA C-3′ and 5′-ACC AGC GCA GCG ATA TCG TC-3′ with probe 6FAM-CCG CCA CCA GTT CGC CAT G-TAMRA. TROP2 human and murine gene expression assays Hs00242741_s1 and Mm00498401_s1 were purchased from Applied Biosystems and validated in-house with PCR efficiencies of 91% and 90%, respectively. Both assays were run according to the manufacturer's protocol. Briefly, the qRT-PCR was performed in a 10-µl reaction with 10 ng of cDNA, 2× Universal PCR Master Mix (Applied Biosystems), 75 nM primers, and 150 nM probe. The RT-PCR amplification program consisted of activation at 50C for 2 min followed by denaturation at 95C for 10 min, and 40 cycles of amplification at 95C for 15 sec and 60C for 1 min with fluorescence capture at each step (ABI PRISM 7900HT Sequence Detection Systems, Applied Biosystems). Threshold cycle values (C_T) were determined, using Sequence Detector software version 2.3 (Applied Biosystems) and transformed to $2^{-\Delta CT}$ for relative expression of *TROP2* to *ACTB*.

Cell Lines

The 293T cell line was obtained from ATCC (CRL-11268). 293T is a derivative of the 293 human embryonic kidney cell line that stably expresses the large T antigen of SV40. 293T cells were grown in DMEM high glucose (GIBCO Inc., Billings, MT) supplemented with 10% FBS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 50 µM 2-mercaptoethanol, glutamine, penicillin, and streptomycin.

Ectopic Expression of Trop2 and Western Analysis

Amphotropic retroviruses were generated by insertion of cDNAs encoding human, cynomolgus, or murine Trop2 into the pLV405G lentiviral vector, followed by packaging into the 293MSR cell line (Amgen Inc., Thousand Oaks, CA). After retroviral transduction, 293T cells were selected by FACS analysis based on the expression of a murine CD8α selection marker co-expressed with Trop2.

For Western analysis, 293T cells were lysed in 20 mM Tris-HCl (pH 8) containing 137 mM NaCl, 5 mM EDTA, 10% glycerol, 1% Triton X-100, and protease inhibitor cocktail mix (11-836-170-001; Roche, Indianapolis, IN). Lysates were treated, where indicated in Figure 1A, left panel, with a protein deglycosylation mix (NEB #P6039) that cleaves both N and O-linked glycans. Twenty µg of total cell lysate was

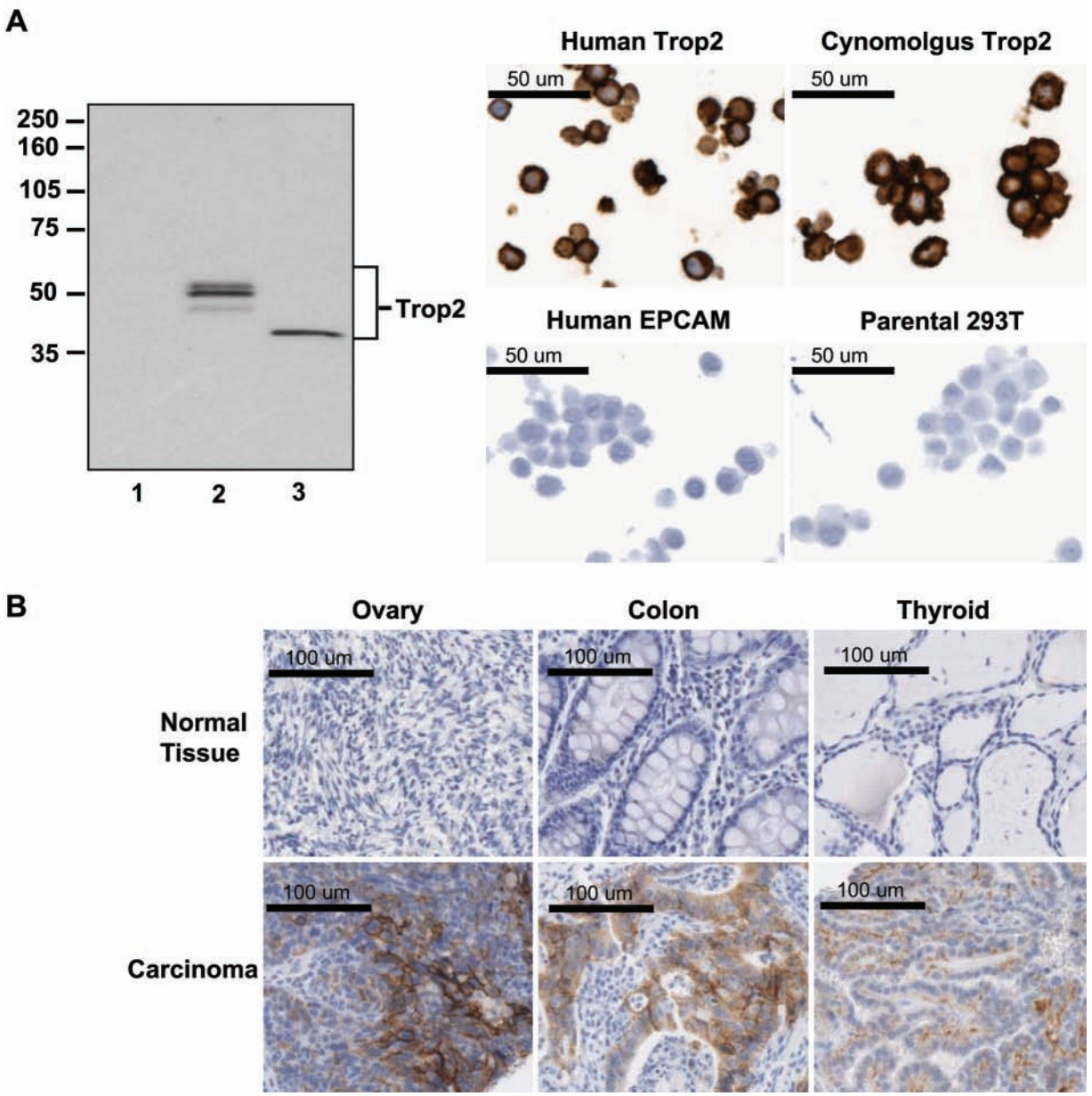

Figure 1. IHC analysis of human Trop2 protein expression. (A) Characterization of IHC antibody specificity. Left panel: MOv16 anti-human Trop2 monoclonal antibody detects bands corresponding to the predicted molecular weight of Trop2 in Western blots of untreated and deglycosylase-treated human Trop2-transduced 293T cells (lanes 2 and 3, respectively) but not parental 293T cells (lane 1). Right panel: MOv16 stains human and cynomolgus Trop2-transduced 293T cells but not human epithelial-specific cell adhesion molecule (EpCAM)-transduced or parental 293T cells. (B) Trop2 is highly expressed on human ovarian, colonic and thyroid carcinomas, but is not detected on the corresponding normal tissues.

loaded per lane of SDS-PAGE gel and transferred to nitrocellulose. The membrane was blocked with 5% skim milk in TBS and incubated with 1 μg/ml of MOv16 mouse IgG1 anti-human Trop2 (ALX-804-650-C100, Enzo Life Sciences, Plymouth Meeting, PA) in TBS/0.05% Tween-20 overnight, followed by sheep anti-mouse IgG-HRP (PA1-28623; Thermo Fisher Scientific, Rockford, IL). Immunoreactive bands were visualized by enhanced chemiluminescence detection.

Immunohistochemistry

Table ST1 contains a complete list of human and animal samples used in IHC assays. IHC for human Trop2 expression was performed on FDA804-1 and FDA804-2 human multi-tissue arrays (US Biomax Inc., Rockville, MD), which included samples of 30 different normal organs as well as samples of a wide variety of tumor types from 30 different

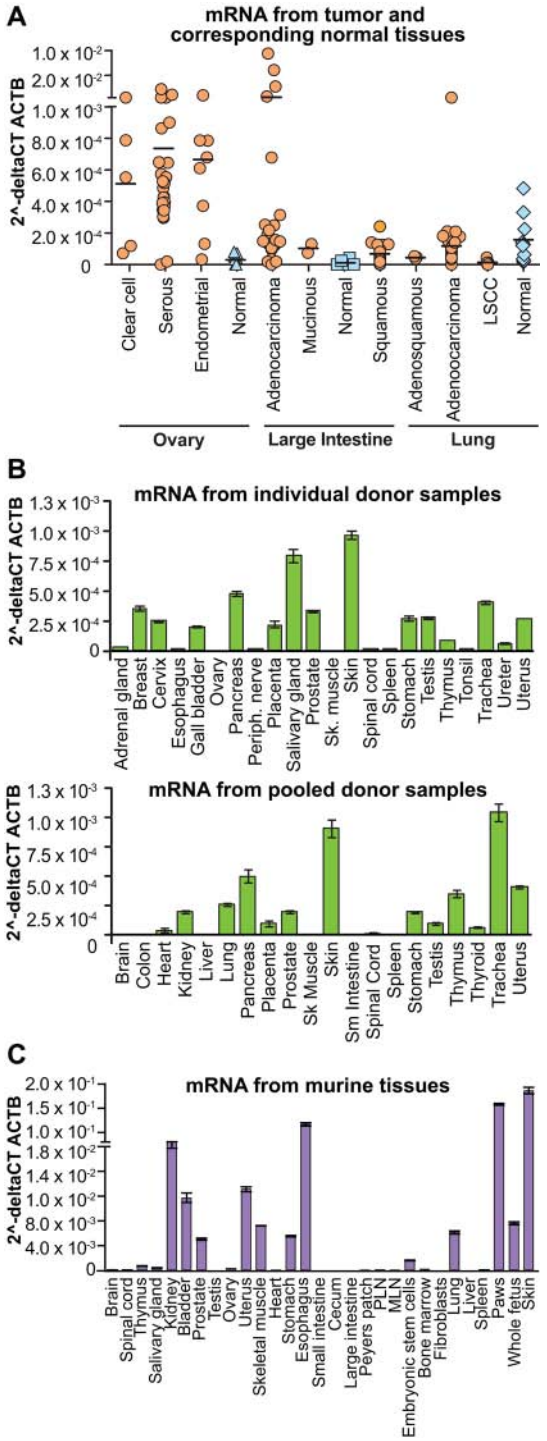

Figure 2. Quantitative PCR analysis of Trop2 message expression in human and mouse. (A) Trop2 is overexpressed by human ovarian and intestinal tumors relative to the corresponding normal tissue. Expression for individual samples and means are shown. (B) Trop2 expression by normal human tissues. Trop2 expression from single patient samples (top panel) and from mRNA pooled from 10 or more patients per sample (bottom panel), relative to housekeeping gene, *ACTB*, is shown. The mean and standard deviation from triplicate samples are indicated. (C) Trop2 expression in normal murine tissues. The mean and standard deviation from triplicate samples are indicated.

organs. For each normal organ type, the arrays included samples from three individuals. Additionally, samples of 55 tumors from different patients were represented. The human Trop2 IHC assay was used to examine expression in samples of eye from three cynomolgus monkeys.

The following monoclonal antibodies were used for the anti-human Trop2 IHC assay at 5 µg/ml final concentration: (1) MOv16 mouse IgG1 anti-human Trop2 (ALX-804-650-C100, Enzo Life Sciences), (2) AMG1 rat IgG1 anti-human Trop2 (Amgen Inc.), and (3) MOPC-31C mouse IgG1 isotype control (550615, BD Pharmingen, Franklin Lakes, NJ). Formalin-fixed paraffin-embedded tissues samples were cut in 4- to 6-µm sections, deparaffinized, and subjected to antigen retrieval using Diva Decloaker (Biocare Medical Inc., Concord, CA) followed by peroxidase and protein blocking (S2001 and X0909, Dako, Denmark). For the anti-human Trop2 assay, a goat anti-mouse EnVision antibody was used (K4001, Dako). Antibody binding was visualized using DAB chromogen (K3466, Dako). Initial staining on controls demonstrated identical results with both anti-human antibodies, so only the MOv16 antibody was used on the human tissue screen and is shown in figure images.

IHC for murine Trop2 expression was performed on a panel of 40 normal mouse organs. Samples from two different individual mice were examined for each organ. Monoclonal antibodies (1) S-19F rat IgG2b raised against the extracellular domain of murine Trop2 (sc-80405, Santa Cruz, CA), (2) TACSTD2 rat IgG2b anti-murine Trop2 (MAB1122, R&D Systems, Minneapolis, MN), and (3) A95-1 rat IgG2b isotype control (553987, BD Pharmingen) were used at 5 µg/ml. For the anti-mouse Trop2 assay, a rabbit anti-rat linker polyclonal antibody (BA-4001, Vector Laboratories Inc., Burlingame, CA) was used, followed by goat anti-rabbit EnVision antibody (K4003, Dako) and visualization with DAB chromogen. Both anti-mouse Trop2 antibodies were used to assay the mouse tissue panel with identical results. Figure 4 shows the IHC stains using the S-19F antibody.

Results

Quantitative mRNA Analysis

To quantitate and compare expression of Trop2 across a panel of normal and tumor tissues, an assay was used that detects human Trop2, but not EpCAM in qRT-PCR experiments. We first analyzed Trop2 expression in ovarian and colonic tissues to determine whether we could reproduce literature reports. We found that Trop2 message is highly expressed in the majority of clear cell, serous, and endometrial ovarian carcinoma tissues examined compared with normal ovary (Figure 2A). Trop2 is also overexpressed in colon adenocarcinomas compared with normal colon (Figure 2A). In contrast to ovarian and colonic tumors, Trop2 is not upregulated on the majority of lung tumors

compared with normal lung (Figure 2A) or on esophageal or head and neck tumors compared with normal esophageal and head and neck tissue (data not shown). Our findings of Trop2 overexpression in ovarian carcinoma and colorectal cancers are consistent with literature reports (Ohmachi et al. 2006; Bignotti et al. 2010).

We observed that in addition to ovarian and colonic tumors, Trop2 message is expressed in a large number of normal human tissues including breast, cervix, gall bladder, kidney, lung, pancreas, placenta, prostate, salivary gland, skin, stomach, testis, thymus, trachea, and uterus (Figure 2B). The Trop2 mRNA expression pattern in a panel of tissues collected from single donors (Figure 2B, top panel), is consistent with the expression observed in a panel of tissue mRNAs from pooled samples (Figure 2B, lower panel). Although Trop2 is highly expressed on many ovarian and colonic tumors (Figure 2A), several essential normal tissues including skin, trachea, pancreas, and kidney also express high levels of Trop2 transcripts (Figure 2B).

Similar to human Trop2, murine Trop2 message is widely expressed across normal tissues, including bladder, uterus, kidney, lung, and skin (Figure 2C). Expression of murine Trop2 mRNA in kidney and lung has been previously reported (El Sewedy et al. 1998). In contrast to human, Trop2 message is also highly expressed in murine esophageal and skeletal muscle tissues (Figure 2C). The broad and conserved expression of Trop2 in human and mouse suggests that Trop2 may play an important role in normal tissue homeostasis. Further studies are required to understand the function and regulation of Trop2 expression in different tissues.

Trop2 IHC

The specificity of MOv16 anti-human Trop2 antibody was confirmed by Western blotting and IHC staining of Trop2-transduced 293T cells (Figure 1A) and by flow cytometry of Trop2 small interfering RNA knockdown in cells naturally expressing Trop2 (data not shown). In Western blots, MOv16 recognized bands at ~48 kDa, the predicted molecular weight (MW) of glycosylated Trop2, in Trop2-transduced, but not parental 293T cells (Figure 1A, left panel, lanes 1 and 2). These bands represent different glycosylation states of Trop2 because upon treatment with a protein deglycosylase mix that cleaves N and O-linked glycans, Trop2 ran as a single band of ~40 kDa MW (Figure 1A, left panel, lane 3). In IHC staining, MOv16 binds to cells transfected with human and cynomolgus Trop2, but not to parental 293T cells or cells transfected with murine Trop2 or human EpCAM (Figure 1A, right panel). Human Trop2 shares approximately 98%, 80%, and 50% amino acid identity with cynomolgus Trop2, murine Trop2, and human EpCAM, respectively.

Consistent with Trop2 mRNA expression (Figure 2A) and previous reports (Ohmachi et al. 2006; Bignotti et al. 2010), Trop2 protein is overexpressed in ovarian and colonic carcinomas with little expression detected in the corresponding normal tissues (Figure 1B). Trop2 is also upregulated on thyroid carcinoma compared with normal thyroid tissue (Figure 1B). Additionally, expression was seen on carcinomas of the breast, cervix, esophagus, lung, pancreas, prostate, stomach, urinary bladder, and uterus (Figure 3 and Table 1), indicating that many epithelial neoplasms express Trop2. In all cases the staining predominantly shows membrane localization sometimes with fainter cytoplasmic signaling.

In contrast to normal ovarian, colonic, and thyroid tissues where Trop2 protein is not detected (Figure 1B), Trop2 is strongly expressed on several normal human tissues including the stratified squamous epithelium of the cervix (Figure 3A), skin (Figure 3B), esophagus, and tonsil crypts (data not shown). Cuboidal or columnar epithelium labels in multiple organs including glands of the breast and prostate, the glands and lining of the uterus, hepatic bile ducts, distal convoluted tubules, and collecting ducts of the kidney as well as glandular and ductular epithelium of the salivary gland and pancreas (Figure 3). Alveolar pneumocytes of the lung and medullary epithelial cells including Hassall's corpuscles in the thymus also express Trop2 (Figures 3A and 3B). In all cases the staining is predominantly localized to the cell membrane sometimes with concurrent fainter cytoplasmic signal. Higher magnification images of select normal and tumor tissues further demonstrate the Trop2 staining pattern (Figure 3C). The Trop2 protein expression pattern (summarized in Table 1) is consistent with the mRNA expression (Figure 2). The intensity of staining on many normal tissues is similar to the corresponding tumor tissue (Figure 3A for examples) and to that observed on ovarian, colonic, and thyroid tumors (Figure 1B).

The S-19F anti-murine Trop2 antibody was used to evaluate Trop2 expression on a broad panel of normal mouse organs. S-19F binds murine Trop2-transduced 293T cells but not parental 293T cells (Figure 4A). Trop2 is strongly expressed on many murine epithelial tissues with the same predominant localization to the cell membrane as was seen in human tissue. Examples of staining on murine cervix, lung, breast, uterus, salivary gland, kidney, thymus, skin, pancreas, and thyroid tissues are shown in Figure 4B. Murine Trop2 message (Figure 2C) and protein expression (summarized in Table 1) overlapped in the tissues tested.

In humans, mutations in Trop2 lead to GDLD, a disorder characterized by amyloid deposits in the cornea of the eye leading to blindness (Tsujikawa et al. 1999). Here, we demonstrate that Trop2 is strongly expressed on the membrane of corneal epithelia cells in the murine eye (Figure 4C). Although samples of human eye were not available, the

Table 1. Summary of Trop2 Protein Expression in Human, Murine, and Tumor Tissues

Human normal tissue expression	**Mouse normal tissue expression**
Stratified squamous epithelium	Stratified squamous epithelium
• Cervix	• Cervix
• Esophagus	• Cornea
• Skin	• Esophagus
• Tonsil-crypt epithelium	• Nonglandular stomach
Cuboidal and columnar epithelium	• Skin
• Breast	• Tongue
• Kidney	Cuboidal and columnar epithelium
• Liver (bile duct epithelium)	• Epididymis
• Pancreas (exocrine)	• Kidney
• Prostate	• Lung bronchi and bronchioles
• Salivary gland	• Mammary gland
• Uterus	• Oviduct
Lung (weak, no bronchioles in samples)	• Salivary gland
Thymic medullary epithelial cells (Hassall's corpuscles)	• Thyroid gland
Human tumor tissue expression	• Trachea
Carcinomas of the breast, cervix, colon, esophagus, lung, ovary, pancreas, prostate, stomach, thyroid, urinary bladder, uterus	• Uterus
Negative human tissues	Transitional epithelium of the ureter and urinary bladder
Brain, bone marrow, colon, heart, intestine, muscle, nerve, ovary, pituitary, spleen, testis, thyroid	Thymic medullary epithelial cells
	Negative mouse tissues
	Adrenal gland, aorta, brain, bone marrow, cecum, colon, duodenum, heart, ileum, jejunum, liver, lymph node, muscle, ovary, pancreas, seminal vesicles, spleen, spinal cord, testis

consistency of this expression across species is demonstrated by identical expression in the cornea of the cynomolgus monkey (Figure 4D). IHC assays using isotype control antibodies were negative in these tissues (Figure 4D).

The Trop2 protein expression pattern is very similar across human and murine tissues. Examples of similarities are shown in kidney, thymus, and skin (Figures 3B and 4B). A few species differences were noted. In particular, human pancreas expresses Trop2 (Figure 3B) whereas murine pancreas is negative (Figure 4B), and Trop2 is detected on murine (Figure 4B) but not human thyroid tissue (Figure 1B). Alveolar pulmonary epithelial cells do not show signal in mouse (Figure 4B) but are positive in human lung (Figure 3A). Staining is more widespread in human salivary gland, whereas it is restricted to the ducts in murine salivary gland (data not shown). All organs/tissues examined are listed in Table ST1, and a summary of Trop2 protein expression in human, murine, and tumor tissues is given in Table 1.

Discussion

Trop2 overexpression on several types of carcinomas and association with tumor invasiveness and poor prognosis (Bignotti et al. 2010; Fong et al. 2008b) render Trop2 a potentially attractive target for antibody-based therapy. A major risk with this approach is the potential toxicity to healthy tissues that normally express the targeted antigen. Trop2 is reported to be expressed in various normal human tissues (Lipinski et al. 1981; Cordon-Cardo et al. 1984; Fradet et al. 1984; Klein et al. 1987; Alberti et al. 1992) and putative prostate cancer stem cells (Goldstein et al. 2008; Trerotola et al. 2010). However, an extensive and uniform evaluation of Trop2 expression in normal tissues remains to be done. Here, we analyzed Trop2 message and protein expression in human, murine and tumor tissues and compared expression in normal and tumor tissues.

Trop2 message is detected in many normal human tissues, most notably skin, kidney, prostate, pancreas, breast, cervix, uterus, lung, and trachea (Figure 2B). Consistent with mRNA findings, the expression of Trop2 protein was previously reported in the distal convoluted tubules and collecting ducts of the kidney; in urothelium; in stratified squamous epithelium of the cervix, esophagus, and skin; and in glands of the prostate and breast by immunofluorescent assays (Fradet et al. 1984; Miotti et al. 1987). We confirmed and expanded these observations to show Trop2 protein expression in epithelium of the tonsil, liver, lung, pancreas, salivary gland, thymus, and uterus (Figure 3 and Table 1).

Aside from a few minor differences such as the thyroid gland and pancreas (Figures 1B and 3B), a similar pattern of Trop2 protein expression is seen in human and mouse. A common theme is membrane localized expression in stratified squamous, cuboidal, and columnar epithelium in both species. Localization of Trop2 to the distal convoluted tubules and collecting ducts in the kidney of both species is also consistent, as is staining in the thymic medullary epithelium and Hassall's corpuscles (Figures 3B and 4B). The wide expression of Trop2 in normal tissues and conservation

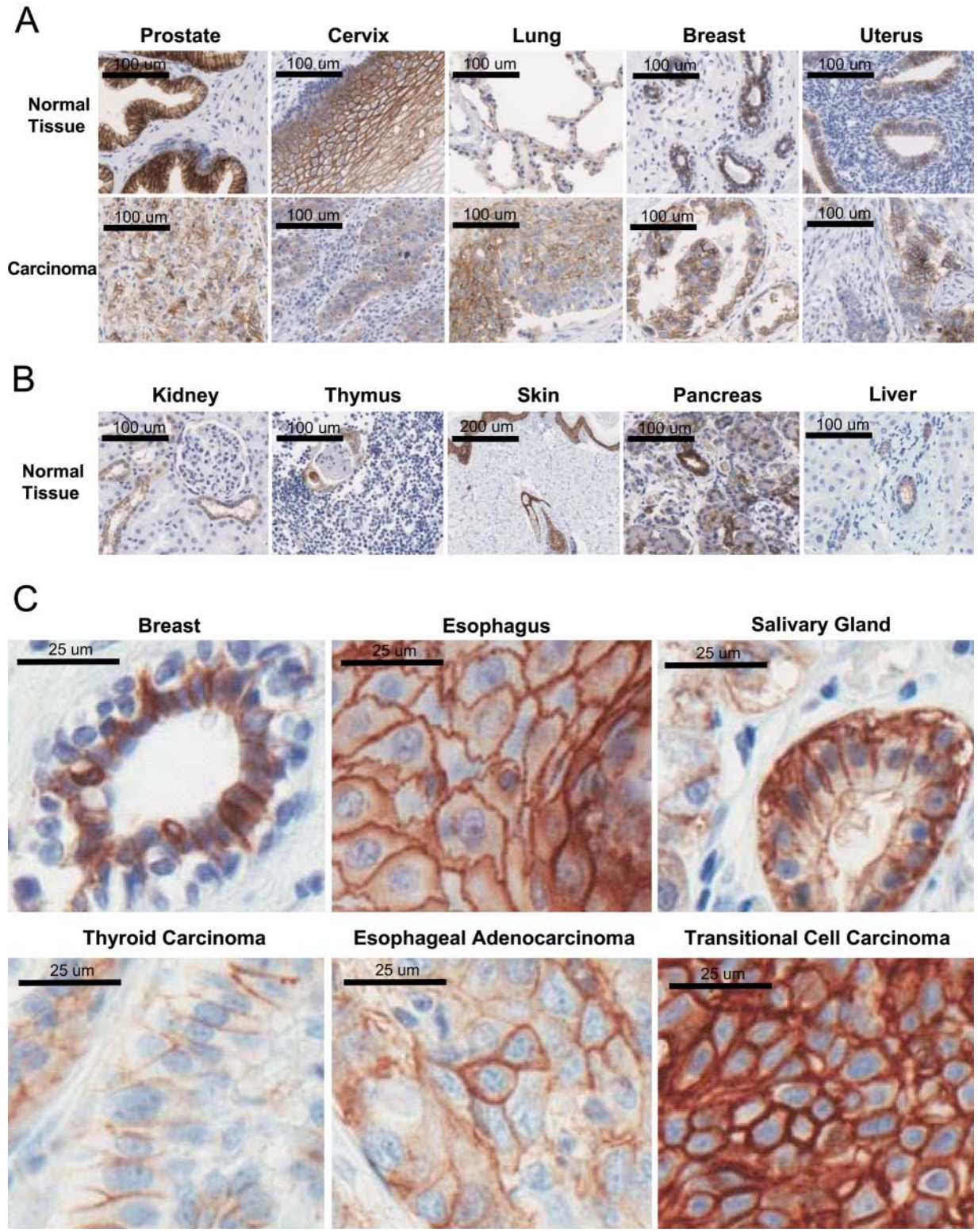

Figure 3. Additional examples of human Trop2 protein expression. (A) Upper panel: Trop2 is highly expressed on many normal tissues including prostate, cervix, lung, breast, and uterus. Lower panel: Trop2 expression on the corresponding tumor tissues. (B) Additional examples of Trop2 expression in human kidney, thymus, skin, pancreas, and liver tissues. (C) High magnification images of selected normal and tumor tissues highlight the subcellular localization pattern of Trop2 staining.

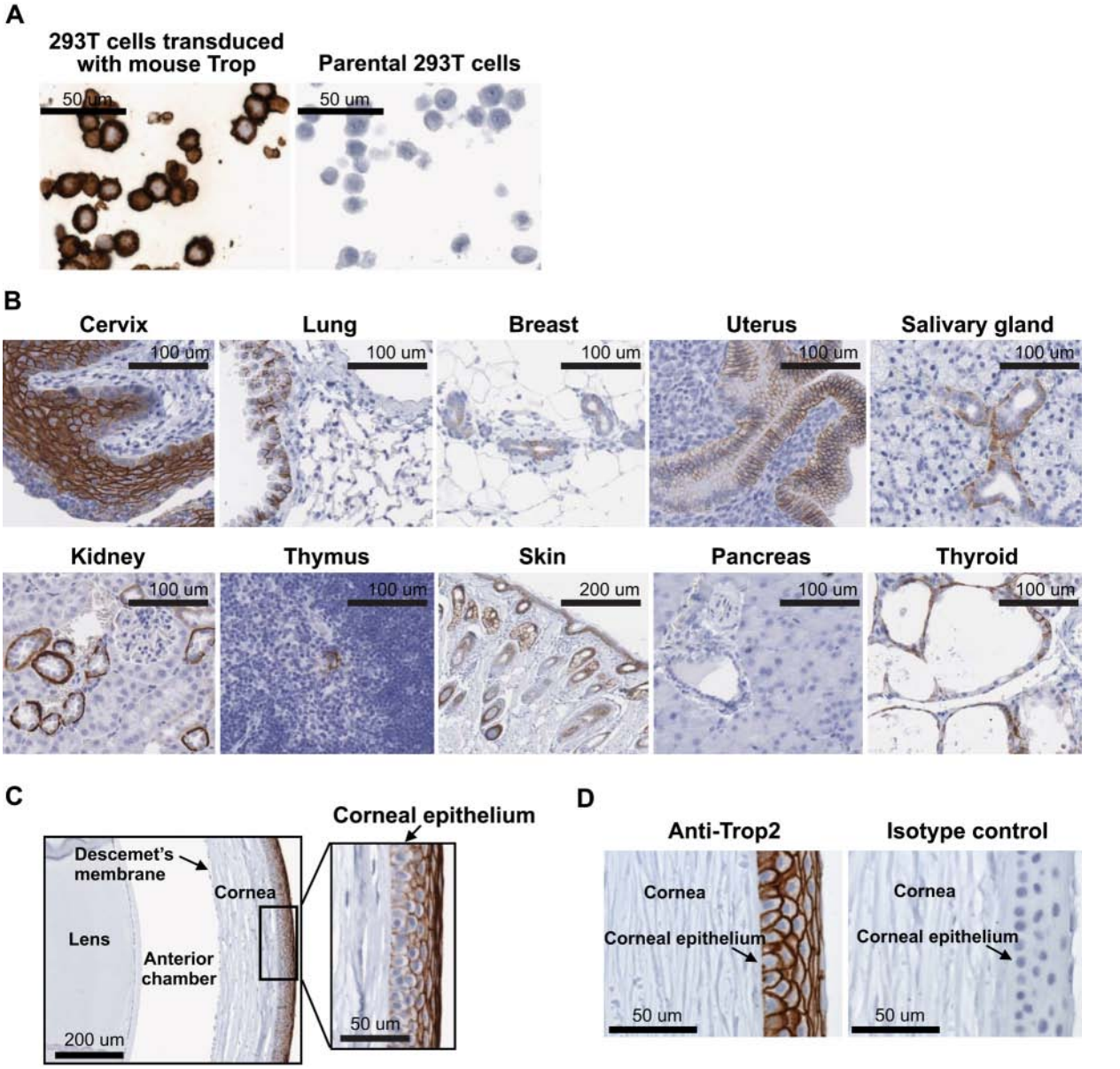

Figure 4. IHC analysis of Trop2 protein expression in murine tissues and cynomolgus eye. (A) S-19F anti-murine Trop2 antibody stains murine Trop2-transduced but not parental 293T cells. (B) Trop2 protein is widely expressed by normal murine tissues. Examples of representative staining of cervix, lung, breast, uterus, salivary gland, kidney, thymus, skin, pancreas, and thyroid tissues are shown. (C) S-19F anti-Trop2 staining of murine eye (D) MOv16 anti-Trop2 staining of cynomolgus eye.

of expression across species suggest that Trop2 may have an important function in normal tissues. EpCAM, the Trop2 paralog, is thought to function as an epithelial cell adhesion molecule (Balzar et al. 2001; Litvinov et al. 1997). Trop2 shares a conserved cysteine-rich region in its extracellular domain that is required for EpCAM-mediated adhesions (Balzar et al. 2001). Whether Trop2 may also play a role in cell adhesion remains to be demonstrated.

IHC revealed Trop2 expression on a number of epithelial neoplasms. In this report, expression is demonstrated in carcinomas of the breast, cervix, colon, esophagus, lung, ovary, pancreas, prostate, stomach, thyroid, urinary bladder, and uterus (Figure 1B, Figure 3, and Table 1). The staining predominantly showed a strong membrane pattern or a mixed membrane and cytoplasmic pattern. Strong expression on ovarian, colonic, and thyroid carcinoma contrasted with little to no expression in the corresponding normal tissue (Figure 1B). However, the level of Trop2 expression in these carcinomas appears to be similar to the level in many normal tissues including prostate, cervix, lung, breast,

uterus, kidney, skin, pancreas and liver, salivary gland, and esophagus (Figure 3), raising the question of whether therapeutics capable of targeting Trop2 on both normal and tumor tissues would have a significant therapeutic window when administered systemically. The expression of Trop2 in many other tumor types including carcinoma of the breast, cervix, esophagus, lung, pancreas, prostate, and uterus was similar in intensity to the corresponding normal tissue (examples shown in Figure 3).

Strong membrane localized Trop2 labeling of the murine corneal epithelium (Figure 4C) is consistent with an important role of Trop2 in the mechanism of the human condition of GDLD. An identical labeling pattern observed in the cynomolgus monkey corneal epithelium (Figure 4D) suggests that this Trop2 localization is consistent across species. GDLD is caused by mutations in Trop2 and is characterized by increased permeability of the corneal epithelium (Kinoshita et al. 2000) and deposition of amyloid material in the subepithelial space of the cornea (Tsujikawa et al. 1999). The strong expression of Trop2 in the corneal epithelium suggests that particular attention will have to be given to potential eye toxicities with therapeutics that target Trop2 and are delivered systemically.

In summary, our findings of wide Trop2 expression indicate that the successful development of therapeutic agents to target Trop2 might require strategies that target the delivery of the therapeutic agent to the malignant tissue in order to minimize potential toxicities to normal tissues that also express high levels of Trop2. Of most concern as potential target liabilities are (1) Trop2 expression in tissue covering large surface areas such as the skin, oral mucosa, and esophagus; (2) Trop2 expression in functionally essential organs such as the pancreas, kidney, and liver; and (3) Trop2 expression in the cornea with the potential mechanistic role of Trop2 in maintaining vision. No studies on the potential efficacy and toxicity associated with targeting Trop2 in humans are available. However, clinical studies with high-affinity ING-1 human-engineered (Xoma Inc., Berkeley, CA) and 3622W94 humanized (GlaxoWellcome Inc., Brentford, UK) monoclonal antibodies targeting EpCAM in patients with adenocarcinomas describe cases of acute pancreatitis (De Bono et al. 2004) and elevation in pancreatic enzymes (Saleh, Posey, et al. 1998; Saleh, LoBuglio, et al. 1998). Similar to our findings with Trop2, EpCAM protein expression in normal pancreatic tissue is reported (Takiyama et al. 1989), suggesting that administration of high-affinity antibodies to Trop2 may pose similar concerns.

Acknowledgments

The authors thank Ian Foltz for critical reading of the manuscript, Kurt Edelmann and Ian Foltz for helpful discussions, and Larry Woody for performing the IHC assays.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the authorship and publication of this article.

Funding

The author(s) received no financial support for the research and authorship of this article.

References

Alberti S, Miotti S, Stella M, Klein CE, Fornaro M, Menard S, Colnaghi M. 1992. Biochemical characterization of Trop-2, a cell surface molecule expressed by human carcinomas: formal proof that the monoclonal antibodies T16 and MOv-16 recognize Trop-2. Hybridoma. 11:539-545.

Balzar M, Briaire-De Bruijn IH, Rees-Bakker HAM, Prins FA, Helfrich W, De Leij L, Riethmuller G, Alberti S, Warnaar SO, Fleuren GJ, et al. 2001. Epidermal growth factor-like repeats mediate lateral and reciprocal interactions of Ep-CAM molecules in homophilic adhesions. Mol Cell Biol. 21:2570-2580.

Bignotti E, Todeschini P, Calza S, Falchetti M, Ravanini M, Tassi RA, Ravaggi A, Bandiera E, Romani C, Zanotti L, et al. 2010. Trop-2 overexpression as an independent marker for poor overall survival in ovarian carcinoma patients. Eur J Cancer. 46:944-953.

Cordon-Cardo C, Bander NH, Fradet Y, Finstad CL, Whitmore WF, Lloyd KO, Oettgen HF, Melamed MR, Old LJ. 1984. Immunoanatomic dissection of human urinary tract monoclonal antibodies. J Histochem Cytochem. 32:1035-1040.

Cubas R, Zhang S, Li M, Chen C, Yao Q. 2010. Trop2 expression contributes to tumor pathogenesis by activating the ERK MAPK pathway. Mol Cancer. 9:253-265.

De Bono JS, Tolcher AW, Forero A, Vanhove GFA, Takimoto C, Bauer RJ, Hammond LA, Patnaik A, White ML, Shen S, et al. 2004. ING-1, a monoclonal antibody targeting Ep-CAM in patients with advanced adenocarcinomas. Clin Cancer Res. 10:7555-7565.

El Sewedy T, Fornaro M, Alberti S. 1998. Cloning of the murine Trop2 gene—conservation of a Pip2-binding sequence in the cytoplasmic domain of Trop-2. Intl J Cancer. 75:324-330.

Fong D, Moser P, Krammel C, Gostner JM, Margreiter R, Mitterer M, Gastl G, Spizzo G. 2008. High expression of TROP2 correlates with poor prognosis in pancreatic cancer. Br J Cancer. 99:1290-1295.

Fong D, Spizzo G, Gostner JM, Gastl G, Moser P, Krammel C, Gerhard S, Rasse M, Laimer K. 2008. TROP2: a novel prognostic marker in squamous cell carcinoma of the oral cavity. Mod Pathol. 21:186-191.

Fornaro M, Dell'Arciprete R, Stella M, Bucci C, Nutini M, Capri MG, Alberti S. 1995. Cloning of the gene encoding Trop-2, a cell-surface glycoprotein expressed by human carcinomas. Intl J Cancer. 62:610-618.

Fradet Y, Cordon-Cardo C, Thomson T, Daly ME, Whitmore WFJ, Lloyd KO, Melamed MR, Old LJ. 1984. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. Proc Natl Acad Sci U S A. 81:224-228.

Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP, Witte ON. 2008. Trop2 identifies a subpopulation of murine and

human prostate basal cells with stem cell characteristics. Proc Natl Acad Sci U S A. 105:20882-20887.

Guerra E, Trerotola M, Dell'Arciprete R, Bonasera V, Palombo B, El-Sewedy T, Ciccimarra T, Crescenzi C, Lorenzini F, Rossi C, et al. 2008. A bicistronic CYCLIN D1-TROP2 mRNA chimera demonstrates a novel oncogenic mechanism in human cancer. Cancer Res. 68:8113-8121.

Kinoshita S, Nishida K, Dota A, Inatomi T, Koizumi N, Elliot A, Lewis D, Quantock A, Fullwood N. 2000. Epithelial barrier function and ultrastructure of gelatinous drop-like corneal dystrophy. Cornea. 19:551-555.

Klein CEK, Cordon-Cardo C, Soehnchen R, Cote RJ, Oettgen HF, Eisinger M, Old LJ. 1987. Changes in cell surface glycoprotein expression during differentiation of human keratinocytes. J Invest Dermatol. 89:500-506.

Lipinski M, Parks DR, Rouse RV, Herzenberg LA. 1981. Human trophoblast cell-surface antigens defined by monoclonal antibodies. Proc Natl Acad Sci U S A. 78:5147-5150.

Litvinov SV, Balzar M, Winter MJ, Bakker HAM, Briaire-De Bruijn IH, Prins F, Fleuren GJ, Warnaar SO. 1997. Epithelial cell adhesion molecule (Ep-CAM) modulates cell-cell interactions mediated by classic cadherins. J Cell Biol. 139:1337-1348.

Miotti S, Canevari S, Menard S, Mezzanzanica D, Porro G, Pupa SM, Regazzoni M, Tagliabue E, Colnaghi MI. 1987. Characterization of human ovarian carcinoma-associated antigens defined by novel monoclonal antibodies with tumor-restricted specificity. Intl J Cancer. 39:297-303.

Muhlmann G, Spizzo G, Gostner J, Maier H, Moser P, Gastl G, Zitt M, Muller HM, Margreiter R, Ofner D, et al. 2009. TROP2 expression as prognostic marker for gastric carcinoma. J Clin Pathol. 62:152-158.

Munz M, Baeuerle PA, Gires O. 2009. The emerging role of EpCAM in cancer and stem cell signaling. Cancer Res. 69:5627-5629.

Nakatsukasa M, Kawasaki S, Yamasaki K, Fukuoka H, Matsuda A, Tsujikawa M, Tanioka H, Nagata-Takaoka M, Hamuro J, Kinoshita S. 2010. Tumor-associated calcium signal transducer 2 is required for the proper subcellular localization of claudin 1 and 7. Am J Pathol. 177:1344-1355.

Ohmachi T, Tanaka F, Mimori K, Inoue H, Yanaga K, Mori M. 2006. Clinical significance of TROP2 expression in colorectal cancer. Clin Cancer Res. 12:3057-3063.

Ripani E, Sacchetti A, Corda D, Alberti S. 1998. Human TROP-2 is a tumor-associated calcium signal transducer. Intl J Cancer. 76:671-676.

Saleh M, Posey J, Khazaeli Mea. 1998. Phase I trial testing multiple doses of humanized monoclonal antibody (MAb) 3622W94 [abstract]. Proc Am Soc Clin Oncol. 17:436.

Saleh MN, LoBuglio AF, Posey JA, Ostroff JB, Khor SP, Thurmond LM, Bigley JW. 1998. Phase I study of different schedules of the humanized anti-EGP40 mAb 3622W94 in patients with antigen-expressing epithelial tumors [abstract]. Cancer Biother Radiopharm. 13:54.

Sebastian M, Kiewe P, Schuette W, Brust D, Peschel C, Schneller F, Ruehle KH, Nilius G, Ewert R, Lodziewski S, et al. 2009. Treatment of malignant pleural effusion with the trifunctional antibody catumaxomab (Removab) (Anti-EpCAM x Anti-CD3): results of a phase 1/2 study. J Immunother. 32:195-202.

Takiyama Y, Tempero MA, Takasaki H, Onda M, Tsuchiya R, Buchler M, Ness M, Colcher D, Schlom J, Pour PM. 1989. Reactivity of CO17-1A and B72.3 in benign and malignant pancreatic diseases. Hum Pathol. 20:832-838.

Trerotola M, Rathore S, Goel HL, Li J, Alberti S, Piantelli M, Adams D, Ziang Z, Languino LR. 2010. Trop-2 and alpha-2beta1 integrin surface receptors as markers of putative human prostate cancer stem cells. Am J Transl Res. 2:135-144.

Tsujikawa M, Kurahashi H, Tanaka T, Nishida K, Shimomura Y, Tano Y, Nakamura Y. 1999. Identification of the gene responsible for gelatinous drop-like corneal dystrophy. Nat Genet. 21:420-423.

Wang J, Day R, Dong Y, Weintraub SJ, Michel L. 2008. Identification of Trop-2 as an oncogene and an attractive therapeutic target in colon cancers. Mol Cancer Ther. 7:280-285.

Weber FL, Bable J. 1980. Gelatinous drop-like dystrophy; a form of primary corneal amyloidosis. Arch Opthamol. 98:144-148.

Zanna P, Trerotola M, Vacca G, Bonasera V, Palombo B, Guerra E, Rossi C, Lattanzio R, Piantelli M, Alberti S. 2007. Trop-1 are conserved growth stimulatory molecules that mark early stages of tumor progression. Cancer. 110:452-464.