Loss of Trop2 Promotes Carcinogenesis and Features of Epithelial to Mesenchymal Transition in Squamous Cell Carcinoma

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Abstract
Trop2, an oncogenic cell surface protein under investigation as a therapeutic target, is commonly overexpressed in several epithelial tumor types yet its function in tumor biology remains relatively unexplored. To investigate the role of Trop2 in epithelial carcinogenesis, we generated Trop2⁻/⁻ mice, which are viable and possess a normal lifespan. Contrary to expectations, Trop2 loss fails to suppress keratinocyte transformation. Instead, ras-transformed Trop2⁻/⁻ keratinocytes preferentially pass through an epithelial to mesenchymal transition (EMT) and form tumors with spindle cell histology. Furthermore, Trop2 loss renders Arf-null mice susceptible to the formation of biphasic sarcomatoid carcinomas containing both squamous and spindle cell components upon carcinogen exposure in an otherwise skin cancer–resistant strain (C57BL/6). Immortalized keratinocytes derived from Trop2⁻/⁻ Arf⁻/- mice exhibit enhanced proliferative and migratory capacity as well as increased activation of mitogen-activated protein kinase and Src prior to transformation. The clinical relevance of these findings was supported by studying the molecular epidemiology of Trop2 in primary head and neck squamous cell carcinomas. This analysis revealed that Trop2 mRNA levels are decreased in a subset of tumors with features of EMT, and total loss of Trop2 protein expression is observed in the spindle cell component of sarcomatoid carcinomas. Therefore, while previous studies have emphasized the potential importance of Trop2 gain of function, these results uncover a role for Trop2 loss in tumorigenesis and the mesenchymal transdifferentiation observed in a subset of squamous cell carcinomas. Mol Cancer Res; 9(12); 1686–95. ©2011 AACR.

Introduction
Trop2 (TACSTD2, M1S1) is a single-pass transmembrane protein expressed primarily in epithelial cells (1) and is often overexpressed in various epithelial tumor types (2–6). We previously reported that Trop2 overexpression has transforming activity, whereas loss of function in colon cancer cells suppresses their tumorigenicity (7). More recently, Trop2 overexpression was shown to strongly induce mitogen-activated protein kinase (MAPK) activity and metastasis in pancreatic cancer cells (8). As such, anti-Trop2 antibodies are under investigation as cancer therapeutics in the preclinical setting (9, 10). Intriguingly, Trop2 expression was found to mark prostate stem cells that are preferentially susceptible to transformation (11). These diverse epidemiologic and functional (12) data point to an important role for Trop2 in tumorigenesis and as a possible treatment target, yet its function(s) remains obscure. Studies on gelatinous drop-like corneal dystrophy (GDCD), a rare form of congenital blindness caused by Trop2 loss-of-function mutations (1, 13, 14), have revealed a role for Trop2 in modulating cell adhesion in the cornea. However, despite this increasing interest in Trop2 as a therapeutic target, its role in normal biology or neoplastic progression is poorly understood.

The epithelial to mesenchymal transition (EMT) consists of a series of cell biological and biochemical changes that endow normal epithelial cells with the plasticity required for body patterning and tissue differentiation. Acquisition of a mesenchymal phenotype by epithelial cells is regulated at multiple levels and is characterized by the loss of E-cadherin–mediated cell–cell adhesion, decreased cell–matrix adhesion, and gain of mesenchymal markers such as vimentin and fibronectin (15–18). An EMT-like profile is considered to be a characteristic of aggressive cancers. However, definitive
identification of EMT in primary human tumors has been difficult, possibly because the transition may be restricted—both spatially and temporally—to a subset of cells within the tumor mass (19). Notably, some epithelial tumors contain areas of pure mesenchymal histology and possess the molecular features of EMT more uniformly. Sarcomatoid carcinoma is one specific example. These tumors are a distinct variant of squamous cell carcinoma that frequently originate in the epithelia of the upper aerodigestive tract (12, 20), display spindle cell histology, and typically lose epithelial gene expression consistent with mesenchymal differentiation. Notably, sarcomatoid carcinoma can be biphasic and exhibit discreet regions of squamous and spindle cell histology. In addition, microarray analyses have resulted in the identification of subtypes of epithelial tumors that do not histologically appear as spindle cells yet possess a gene expression signature suggestive of an epithelial to mesenchymal transition (21, 22).

Given the reported overexpression of Trop2 in various epithelial tumors, we sought to examine its role in epithelial carcinogenesis by deriving Trop2 knockout mice and report the generation of a viable Trop2-null strain. Contrary to expectations, we show that Trop2 loss promotes the development of EMT during ras-mediated transformation of primary keratinocytes. In addition, Trop2 loss increases susceptibility to the development of biphasic sarcomatoid carcinomas of the skin in response to carcinogens. Accordingly, in head and neck squamous cell carcinomas (HNSCC), we find that Trop2 expression is decreased in tumors possessing an EMT-like signature and completely absent in sarcomatoid variants. Collectively, these data implicate Trop2 loss in tumorigenesis in keratinocytes and in the promotion of EMT in a subset of squamous cell carcinomas.

**Materials and Methods**

**Generation of Trop2<sup>Δ−/−</sup> mice**

Bacterial artificial chromosome (BAC) DNA clone RP23-23I10 containing Trop2 locus was obtained from CHORI and was used to construct targeting vector by PCR amplification. The primer sequences used for 5′ homology arm of the targeting construct were 5′-GAT TGA GGA TCC AAT CTC TCC CAG GTC ATC ATA AAC-3′ and 5′-GAT TGA GGA TCC GGT GGG GTG GAG TAG AAT GGA-3′. The primer sequences used for 3′ homology arm of the targeting construct were 5′-GAT TGA ATC CCA TGG CCA AAG ACT TAA ACG GTT TGA AAT G-3′ and 5′-GAT TGA GGC GCC GCA GAG CCC CTC CGG CTT CTC ACA-3′. The amplified 5′ homology arm (2.6 kb) and 3′ homology arm (3.0 kb) were cloned into the p1338 vector. The targeting vector was linearized with NotI, and gene targeting was conducted following standard procedures in C57BL/6SJ embryonic stem cell line B6/Blu, which was injected into a blastocyst. A pseudopregnant C57BL/6SJ mother was used as the recipient. Embryonic stem cell clones that underwent the correct integration were identified by Southern blotting with a 5′ external probe and 3′ external probe. The primer sequences used for the 5′ external probe were 5′-CTC TAC TCC TAA CCC TAA TCT GCC-3′ and 5′-TAC CAT CCC TAC CT TGT AGT CCA CC-3′. The primer sequences used for the 3′ external probe were 5′-GTC CCT ACT CAA CTC CTT CTC CCG-3′ and 5′-TAG CCA ATT ACA AAA CAG TCA TT CCG C-3′. One embryonic stem cell clone was used to generate chimeras by Washington University Murine Embryonic Stem Cell Core (St. Louis, MO).

**Skin tumor induction**

The backs of 8-week-old male and female mice were shaved and treated with a single application of 7,12-dimethylbenz(a)anthracene (DMBA; 20 μg in 200 μL of acetone; Sigma) followed a week later by twice weekly applications of 12-O-tetradecanoylphorbol-13-acetate (TPA, 12.5 μg in 200 μL of acetone). The number and size of papillomas on each mouse were recorded every week for 36 weeks. Animals were handled according to protocols approved by the Washington University Animal Studies Committee.

**Mouse keratinocyte isolation and manipulation**

Primary epidermal keratinocytes populations were harvested from newborn mice (1 day old). Skin was floated dermis down at 4°C overnight in dispase I (Invitrogen). Afterwards, skin pieces were placed with the dermis side up, and the dermis was peeled off with a forceps. Keratinocytes were isolated by trypsinizing the tissues for an additional 15 minutes at 37°C. Cells were collected, washed in PBS containing 0.5% FBS, and were resuspended and seeded in Eagle’s Minimal Essential Medium (EMEM; Lonza).

**Antibodies and immunoblot analysis**

Cells were lysed in 50 mmol/L Tris, pH 7.4; 150 mmol/L NaCl; 0.5% sodium deoxycholate (DOC); and 0.5% NP-40. Fifty microgram of protein was resolved on a 4% to 15% Tris-HCl gel and transferred to polyvinylidene fluoride membranes. Antibodies used in this study were against Trop2 A650 and AF1122 (against human and mouse, respectively; R&D Systems), E-cadherin (BD Transduction Laboratories), vimentin (BD Pharmpingen), p-Src (Tyr416; Calbiochem and Cell Signaling), tubulin (Abcam), Erk 1/2 and p-ERK 1/2 (Thr202/Tyr204), p-FAK (Tyr397), p-130Cas (Tyr165; Cell Signaling), and H-Ras (Santa Cruz Biotechnology). Image quantification was carried out with ImageJ software (NIH, Bethesda, MD).

**Lentiviral gene transfer and tumorigenicity assays**

Lentiviruses expressing H-rasV12 or mouse Trop2 were produced as previously described (7). Filtered supernatant supplemented with 4 μg/mL polybrene was used to infect freshly isolated mouse skin keratinocytes within 48 hours postplating. Two days after infection, lentivirally transduced cells were harvested, and either 2 × 10<sup>4</sup> cells were injected subcutaneously into the flanks of nude mice. Tumor growth was monitored every 3 days.
Immunohistochemical analysis

The slides were deparaffinized and hydrated, treated in citrate buffer (0.1 mol/L sodium citrate and citric acid, pH 3.0) for 10 minutes in a pressure cooker, and quenched in 3% hydrogen peroxide solution for 10 minutes. Sections were blocked with avidin solution and biotin solution after washing with TBS Tween 20 (TBST) thoroughly. Sections were incubated with anti-Trop2 antibody for 1 hour at room temperature after TBST washing. Sections were incubated with LSAB+System-HRP solutions (Dako) per the manufacturer’s instructions. Color was developed by adding 3,3′-diaminobenzidine (DAB) substrate solution and stopped by washing with water. Mouse tumor slides were counterstained in hematoxylin, dehydrated, cleared, and mounted in Cytoseal. The histology of keratinocyte tumors was quantified by the Olympus Microsuite 5 software package.

Statistical analysis

All statistical tests were 2-sided, and P values of less than 0.05 were considered statistically significant.

Additional methods can be found in Supplementary Material.

Results

Trop2 loss does not result in embryonic lethality and is not required for development in mice

To gain insight into the effects of Trop2 loss of function, we generated a Trop2-null mouse strain by deleting the single exon that encodes the open reading frame using homologous recombination in embryonic stem cells (Fig. 1A–E). Trop2 has been proposed to play a role in development because its expression is high in certain epithelial stem cell compartments (23, 24), and the levels fluctuate widely in response to developmental cues (25). In addition, EpCAM, the gene most closely related to Trop2 (49% homology) is essential in normal life expectancy (Supplementary Figs. S1 and S2 and data not shown).

Induction of tumors with spindle cell histology upon transformation of Trop2−/−-null keratinocytes

To model the effects of Trop2 loss of function on epithelial transformation, we used the 2-hit transformation assay of primary mouse keratinocytes (27) in which ras pathway activation in the context of Arf deletion drives the development of squamous cell carcinomas (28). Primary keratinocytes were isolated from Trop2+/−:Arf+/− and Trop2−/−:Arf−/− mice, infected in vitro with an H-RasV12-expressing lentivirus (Supplementary Fig. S3), and then grafted subcutaneously into the flanks of nude mice. Palpable tumors were noted in both genetic backgrounds after 2 weeks when a cell dosage of 2 × 10⁶ was injected. However, histopathologic examination of the tumors that arose revealed that keratinocytes from Trop2+/−:Arf−/− mice displayed primarily spindle with almost no squamous cell histology. In contrast tumors from Trop2+/−:Arf−/− mice exhibited predominantly squamous cell histology and some spindle cell components (Fig. 2A). Quantification of the percentages of spindle and squamous histology in each tumor with image analysis software confirmed an increase in spindle cell conversion in tumors derived from Trop2−/−:Arf−/− cells compared with those arising from Trop2+/−:Arf−/− cells (Fig. 2B). Analysis of E-cadherin and vimentin in tumors arising from both genotypes revealed the retention of E-cadherin and the absence of vimentin expression in areas of squamous cell histology, whereas spindle cells displayed the opposite pattern, consistent with EMT (Fig. 2C and Supplementary Fig. S4). Strikingly, in areas of spindle cell histology in nominally Trop2 wild-type tumors, Trop2 expression was absent, and a gradient of decreasing expression was evident mirroring the decline in E-cadherin and increase in vimentin staining in transition zones bordering cells that had passed through an EMT (Fig. 2C). These data indicate that Trop2 deficiency facilitates EMT during oncogenic transformation of keratinocytes in vitro. Furthermore, Trop2 loss occurs during transformation associated with mesenchymal transdifferentiation in Trop2 positive cells.
A Role for Trop2 Loss in Carcinogenesis

Trop2 loss promotes tumorigenesis in mice

The ability of Trop2 loss to modify the histologic outcome of an identical set of transforming events at the cellular level raised the question whether Trop2 loss contributes to tumorigenesis. To investigate this possibility, we used a well established model of skin carcinogenesis (DMBA—TPA) that in C57BL/6 mice generates H-Ras mutations and papillomas but rarely invasive cancer (29). The absence of Trop2 did not alter the incidence or natural history of papilloma formation by this protocol (Supplementary Table 1A). However, given the cooperativity between Trop2 and Arf—ras pathway defects observed in keratinocytes, the effect of Trop2 loss on skin cancer development was assessed in Arf-null animals exposed to DMBA—TPA. Over a standard 36-week treatment period, both strains developed papillomas at equivalent rates and sizes (Supplementary Table 1B) and equivalent percentages of mice survived the treatment protocol, some succumbing to the expected lymphoid and nonskin sarcomatoid appearing malignancies. However and most importantly, Trop2 loss in this context was sufficient to promote the formation of skin carcinomas (n = 6/24 in Trop2−/−; Arf−/− mice), whereas none of the Trop2−/−:Arf−/− mice (n = 0/25, P = 0.009, the Fisher exact test) developed cancer (Fig. 3A and B). All 6 tumors were invasive, 5 were classified as sarcomatoid carcinomas, and 1 was found to consist of poorly differentiated high-grade cancer. Interestingly, some squamous cell histology was identified in 3 of the sarcomatoid carcinomas, consistent with their epithelial origin. Accordingly, these squamous cell components expressed E-cadherin, whereas the spindle cells lacked this staining but were positive for vimentin (Fig. 3C). Thus, Trop2 loss, in collaboration with Arf inactivation, increases the susceptibility to skin carcinogenesis and promotes the development of tumors with mesenchymal features.

Immortalized Trop2-null keratinocytes exhibit increased proliferative and migratory capacity

Trop2-null mice display no overt phenotypic abnormalities, yet Trop2 loss can contribute to tumor initiation and
drive progression towards spindle cell histology. These observations suggest that nontransformed Trop2-null cells are more likely to acquire characteristics that can facilitate cancerous growth. To investigate whether such characteristics could be identified, we isolated Trop2\(^{+/+}\)/Arf\(^{-/-}\) and Trop2\(^{-/-}\)/Arf\(^{-/-}\) keratinocytes; the Arf deficient background imparts features of cellular immortalization in vitro (30). Loss of Trop2 did not alter cellular proliferation in standard tissue culture conditions (data not shown), but Trop2\(^{-/-}\)/Arf\(^{-/-}\) keratinocytes displayed a dramatic proliferative advantage in 3-dimensional culture compared with Trop2\(^{+/+}\)/Arf\(^{-/-}\) cells. Measurement of BrdUrd incorporation revealed increased rates of DNA synthesis in Trop2-null cells when grown in 3-dimensional culture, suggesting a proliferative advantage under these more physiologic conditions (Fig. 4A–C). We also compared the migratory

**Figure 4.** Trop2 loss confers a proliferative and migratory advantage in Arf\(^{-/-}\) immortalized keratinocytes. A, photomicrographs of Trop2\(^{+/+}\)/Arf\(^{-/-}\) and Trop2\(^{-/-}\)/Arf\(^{-/-}\) keratinocytes grown on Matrigel for 7 days. 10\(\times\) magnification. B, graph of colony counts 7 days after keratinocyte plating. C, graph of BrdUrd incorporation of keratinocytes grown on Matrigel measured 3 days after plating. \(P = 0.025\), the Student t test. D, photomicrographs of a scratch assay using keratinocytes from Trop2\(^{+/+}\)/Arf\(^{-/-}\) and Trop2\(^{-/-}\)/Arf\(^{-/-}\) mice showing a completely closed wound in Trop2-null cells by 16 hours. E, immunoblot analysis of protein lysates from Trop2\(^{+/+}\)/Arf\(^{-/-}\) and Trop2\(^{-/-}\)/Arf\(^{-/-}\) keratinocytes showing decreased E-cadherin levels in cells lacking Trop2. F, immunoblot analysis of Trop2\(^{-/-}\)/Arf\(^{-/-}\) keratinocytes infected with control (empty vector) or Trop2-expressing lentivirus. Protein levels of Trop2 in Trop2\(^{+/+}\)/Arf\(^{-/-}\) are shown for comparison. G, graph of BrdUrd incorporation of Trop2\(^{-/-}\)/Arf\(^{-/-}\) keratinocytes infected with control or Trop2-expressing virus grown on Matrigel measured 3 days after plating. \(P = 0.049\), the Student t test. BrdUrd incorporation of Trop2\(^{+/+}\)/Arf\(^{-/-}\) keratinocytes is shown for comparison. For BrdUrd experiments, results were normalized to Trop2\(^{+/+}\)/Arf\(^{-/-}\) cells. H, photomicrographs of a scratch assay using keratinocytes from Trop2\(^{-/-}\)/Arf\(^{-/-}\) mice infected with control (EV, empty vector) or Trop2-expressing virus showing retarded migration in Trop2-expressing cells. Arrows point to scattered cells in the unfilled region of the scratch. Results from each experiment are representative of 3 independent tests.
potential of \( Trop2^{+/+}:Arf^{-/-} \) and \( Trop2^{-/-}:Arf^{-/-} \) keratinocytes and found the latter to possess a significantly increased mobility in a 2-dimensional wound-healing assay (Fig. 4D). Next, we examined whether \( Trop2 \) loss was sufficient to promote features of EMT in immortalized keratinocytes. \( Trop2^{-/-}:Arf^{-/-} \) did not acquire a mesenchymal morphology and immunoblot analysis of Twist, Snail, Slug, and fibronectin revealed no detectable alterations in the levels of these proteins compared with wild-type cells. However, E-cadherin protein levels were found to be significantly decreased in the absence of \( Trop2 \) (Fig. 4E and data not shown). Finally, we sought to reconstitute \( Trop2 \) expression in \( Trop2^{-/-}:Arf^{-/-} \) cells with a full-length mouse cDNA in an effort to reverse the observed phenotypic changes. After lentiviral transduction of \( Trop2 \) and antibiotic selection, \( Trop2 \) protein expression was partially restored relative to levels measured in \( Trop2^{+/+}:Arf^{-/-} \) cells (Fig. 4F), and the protein migrated as a doublet, suggesting differential posttranslational modification(s) in this cellular environment. Importantly, expression of \( Trop2 \) in \( Trop2^{-/-}:Arf^{-/-} \) cells was found to be sufficient to reduce (but not normalize) both rates of proliferation in 3-dimensional culture as well as migration in a wound-healing assay relative to those measured in empty vector-transduced cells (Fig. 4G and H). Total E-cadherin levels were not measurably increased (data not shown), possibly owing to the need for higher levels of \( Trop2 \) protein expression to rescue this specific defect in knockout cells. Nevertheless, partial restoration of \( Trop2 \) protein levels is able to measurably reduce the proliferative and migratory advantages identified in \( Trop2 \)-null keratinocytes, observations that point to a causal role for \( Trop2 \) loss in the development of these phenotypes.

To identify \( Trop2 \)-dependent mechanisms underlying the increased susceptibility to tumorigenesis, we examined whether \( Trop2 \) loss could increase the activity of common oncogenic pathways by measuring the phosphorylation status of epidermal growth factor receptor, AKT, extracellular signal–regulated kinase (ERK), and Src. This analysis revealed that keratinocytes derived from \( Trop2^{-/-} \) mice exhibit an increase in MAPK and Src activity, the former showing a dramatic increase in the absence of \( Trop2 \) compared with \( Trop2^{+/+}:Arf^{-/-} \) cells (Fig. 5A). An increase in the activity of epidermal growth factor receptor and AKT was not observed (data not shown). In \( Trop2 \) knockout cells, we also detected an increase in the level of

Figure 5. Activation of MAPK and Src in \( Trop2^{+/+}:Arf^{-/-} \) keratinocytes and tissues. A, immunoblot analysis of immortalized \( Trop2^{+/+}:Arf^{-/-} \) and \( Trop2^{-/-}:Arf^{-/-} \) keratinocytes reveals activation of ERK 1/2 (Thr202/Tyr204), Src (Tyr416), FAK (Tyr397), and \( p130^\text{Cas} \) (Tyr165) in \( Trop2 \)-null cells. B, immunoblot analysis shows reduction in ERK and Src activation in \( Trop2 \)-expressing \( Trop2^{-/-}:Arf^{-/-} \) cells compared with control cells harboring an empty vector. The numbers indicate densitometrically quantified protein levels (normalized to total ERK or Src). C, immunohistochemical staining of p-ERK (Thr202/Tyr204) and p-Src (Tyr416) in papillomas and carcinomas from tissues arising in the denoted genotypes. Arrows and arrowheads point to cells staining positive. Sections were stained with methyl green as a counterstain. Bar is 50 \( \mu \)m.
phosphorylated forms of 2 Src substrates, FAK and p130Cas (Fig. 5A). Importantly and in consonance with the partial phenotypic rescue achieved by Trop2 expression, we observed reduced levels of active MAPK and Src in Trop2<sup>−/−</sup>:Arf<sup>−/−</sup> cells after infection with Trop2-expressing lentivirus (Fig. 5B). To determine whether increased MAPK and Src activity could be identified in the context of Trop2 deficiency in vivo, we conducted an immunohistochemical analysis of papillomas and tumors. Neither MAPK nor Src hyperactivation was found in papillomas arising in Trop2<sup>−/−</sup>:Arf<sup>−/−</sup> mice. In contrast, increased staining of phospho (p)-ERK and phospho (p)-Src was readily detected in papillomas arising in Trop2<sup>−/−</sup>:Arf<sup>−/−</sup> mice (n = 5 per group). In addition, extensive staining was observed in 5 carcinomas examined that developed in the double knockout animals (Fig. 5C).

Interestingly, Src staining was detected primarily in the cytoplasm, a pattern of localization that has previously been associated with poorly differentiated cancer (31). Collectively, these data reveal that Trop2 loss causes an increase in MAPK and Src activation prior to oncogenic transformation.

**Decreased Trop2 levels in squamous cell carcinoma with an EMT gene expression signature and sarcomatoid carcinomas of the head and neck**

Our data showing that loss of Trop2 facilitates EMT in squamous cell tumors in mice prompted us to investigate Trop2 expression in human head and neck cancer. A subset of head and neck cancers exhibit morphologic and/or molecular features of EMT. In addition to sarcomatoid carcinomas of the head and neck, which exhibit mesenchymal markers as well as distinct histopathology suggestive of an EMT, a gene expression signature has previously been reported by us that identifies a subtype of tumors with molecular features of EMT (designated as group 2). Pathologically, these tumors appear as poorly differentiated carcinomas (22). To determine whether decreased Trop2 expression associates with this EMT-like subtype, we queried its expression in our data set that was used to develop this signature (22). Notably, Trop2 expression was found to be decreased in the EMT-like group relative to the other subtypes and normal tonsillar mucosa. Accordingly, reduced Trop2 expression segregates with low E-cadherin and high vimentin expression in this data set (Fig. 6A). Given the relative decrease in these poorly differentiated, EMT-like yet nonsarcomatoid tumors and based on the results from the knockout animal experiments, we suspected that Trop2 expression might be completely undetectable in the spindle cell component of sarcomatoid carcinoma of the head and neck. Immunohistochemical staining of sarcomatoid carcinoma confirmed this possibility. Trop2 expression was detected in areas of squamous cell histology in cancers containing pure squamous cell elements and biphasic sarcomatoid carcinomas. However, in striking contrast, its expression was absent in the spindle cell component of sarcomatoid carcinomas (Fig. 6B and C; Table 1) from various head and neck sites (n = 5 of each histologic group, P = 0.007, the Fisher exact test). Consistent with their mesenchymal state, Trop2-negative sarcomatoid carcinomas also lacked E-cadherin expression, whereas tumors containing Trop2-positive cells with squamous histology expressed high levels (Fig. 6C). Therefore, decreased Trop2 expression is a feature of squamous cell carcinomas that possess molecular and histologic characteristics of EMT.

![Image](http://example.com/image.jpg)

**Figure 6.** Trop2 expression in head and neck cancers. A, association of Trop2 expression levels with intrinsic subtypes of head and neck cancers in silico analysis. Group 2 is the EMT-like subtype consisting of poorly differentiated tumors. B, example of loss of Trop2 staining in a biphasic head and neck cancer. Trop2 is absent in the spindle cell component (sp), whereas nests of squamous cancer cells (sq) stain positive. 10× magnification. C, immunohistochemical staining of Trop2 and E-cadherin showing concomitant loss in spindle cell components, 20× magnification.

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NOTE: Cancers denoted in the left column contain pure squamous cell histology.

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A Role for Trop2 Loss in Carcinogenesis

Discussion

The results presented here were not predicted on the basis of previous epidemiologic and functional studies of Trop2, all of which pointed to a gain-of-function role for this protein in the development of aggressive cancer and tumor susceptibility (2–7, 11, 23, 32–34). As such, we expected that Trop2 ablation would hinder cancer development. Instead, through the use of a novel Trop2 knockout mouse model generated in our laboratory, we uncovered an unpredicted relationship between Trop2 loss and cancer development. In addition, we were able to confirm a relationship between Trop2 loss and an increased progression towards EMT during epithelial transformation. The epidemiologic analysis presented in this report is the first description, to our knowledge, of the association between Trop2 loss and EMT in any primary human tumor and highlights the clinical relevance of our findings. Collectively, these observations reveal a far more complex functional role for this protein in the multistep development of cancer than has been previously suspected.

It has been reported that germline deletion of EpCAM, the closest homologue to Trop2, causes early embryonic lethality in mice (26). In addition, Trop2 expression is elevated in stem cell compartments of the prostate (23) and liver (24), and the pattern of expression is dynamic during development (25). All of these observations suggested a high likelihood that Trop2 deletion would result in abnormalities in development or adulthood, but these animals are viable and lack overt developmental defects. However, a more detailed analysis revealed that Trop2 loss promotes skin cancer in mice lacking Arf, a tumor suppressor gene commonly inactivated in many cancers, including those arising in the head and neck. It is notable that mice lacking this gene are prone to and predominantly develop sarcomas and lymphomas rather than skin tumors (30), which do form when exposed to DMBA as neonates (35). C57BL/6 mice, the strain used in our experiments, are notoriously resistant to the standard TPA–DMBA-mediated carcinogenesis protocol that we used herein. Given that the cancers that develop in Trop2−/−:Arf−/− mice in the C57BL/6 background after TPA–DMBA exposure and the absence of a detectable cancer phenotype in Trop2−/− mice, our results indicate that Trop2 participates in multistep tumorigenesis as a modifier in collaboration with other protumorigenic events.

In this manner, Trop2 loss resembles the effects of Src, whose catalytic activity is frequently elevated in cancers. Owing to its weak transforming ability, Src is thought to facilitate other oncogenic signals rather than function as a dominant oncogene (36). The identification of Src hyperactivation in keratinocytes, papillomas and carcinomas derived from Trop2−/−:Arf−/− mice, and the suppression of this activity by reexpression of Trop2 supports the idea that Trop2-dependent modulation of Src likely contributes to the enhanced tumor susceptibility observed in Trop2−/−:Arf−/− animals. We also found that Trop2 loss causes elevation of MAPK activity, which lies downstream of several oncogenic pathways. We did not assess the relative contributions of these 2 pathways to the observed cellular phenotypes but it is notable that crosstalk between the Src and MAPK pathways is well established. These pathways are commonly linked in adhesion signaling mediated by integrin and hyaluronic acid receptors, suggesting that Trop2 may be a component of one of such module (37).

Strikingly, the squamous cell tumors that arise in Trop2−/−:Arf−/− mice are primarily sarcomatoid or poorly differentiated carcinomas, an observation that is consistent with the primary human tumor data. The ability of Trop2 loss to promote spindle cell histology in primary keratinocyte transformation assays provides additional evidence for a role for Trop2 in modulating the epithelial state. Similar to what has been observed with established inducers of EMT, Trop2 loss–induced mesenchymal transdifferentiation must occur in collaboration with other factors and may be cell type specific (38). This is clear because Trop2 knockout mice are developmentally normal, and the pattern of E-cadherin expression in 2 tissues that we have examined in situ (skin and breast, data not shown) is similar in wild-type and knockout animals. In addition, EMT was not observed in immortalized Trop2−/−:Arf−/−–null keratinocytes, although E-cadherin expression is reduced in these cells. The sarcomatoid histology and E-cadherin loss observed in tumors arising in Trop2−/−:Arf−/− animals are likely to be mediated at least in part through Src activity, a well-established negative regulator of E-cadherin. Activation of the MAPK pathway has also been implicated in the development of aggressive cancer and EMT (15, 39) and may also contribute to Trop2 loss–induced EMT. Intriguingly, gene expression analyses of lung and ovarian cancer cell lines that exhibit features of EMT (as well as those from the head and neck) show strikingly low Trop2 expression than more epithelial lines originating from the same tumors. These results point to the possibility that Trop2 mediates EMT in a broad range of tissues when considering the data presented herein (40–42). Further dissection of the relationship between Trop2, Src, and MAPK activation is the goal of ongoing studies.

The ability of proteins to exert dual (oncogenic or tumor suppressor) functions is well established (43, 44). The observations that both gain and loss of Trop2 function provoke strong phenotypes suggest that this protein possesses potent signaling activities. Given the similarity to EpCAM, which regulates both adhesion (45) and intracellular signaling via cleavage products (46), Trop2 may also possess a pleiotropic mechanism of action. This possibility may reconcile the apparent contradiction that high levels of Trop2 in oral cancers have been observed and associate with a poor outcome in one study (5). As such, studies in the oral mucosa of Trop2 knockout animals are planned to more accurately define the functional role of Trop2 in HNSCC, where EMT is thought to be an important prognostic factor (47).

In summary, while previous studies have highlighted the potential importance of Trop2 gain of function in the
development of aggressive epithelial cancers, the data presented herein identify for the first time a role for Trop2 loss of function in tumorigenesis and mesenchymal transdifferentiation. Ongoing efforts to target Trop2 immunologically (10, 48) will need to take into account the effects of its loss uncovered in this study. Further investigation into Trop2 function through the loss-of-function genetics described in this report should provide insights into EMT and elucidate novel mechanisms of cancer susceptibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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