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SHORT COMMUNICATION

The Trop-2 signalling network in cancer growth

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Our findings show that upregulation of a wild-type Trop-2 has a key controlling role in human cancer growth, and that tumour development is quantitatively driven by Trop-2 expression levels. However, little is known about the regulation of expression of the *TROP2* gene. Hence, we investigated the *TROP2* transcription control network. *TROP2* expression was shown to depend on a highly interconnected web of transcription factors: TP63/TP53L, ERG, GRHL1/Get-1 (grainyhead-like epithelial transactivator), HNF1A/TCF-1 (T-cell factor), SPI1/PU.1, WT (Wilms' tumour)1, GLIS2, AIRE (autoimmune regulator), FOXM1 (forkhead box M1) and FOXP3, with HNF4A as the major network hub. *TROP2* upregulation was shown to subsequently drive the expression and activation of CREB1 (cyclic AMP-responsive-element binding protein), Jun, NF-κB, Rb, STAT1 and STAT3 through induction of the cyclin D1 and ERK (extracellular signal regulated kinase)/MEK (MAPK/ERK kinase) pathways. Growth-stimulatory signalling through NF-κB, cyclin D1 and ERK was shown to require an intact Trop-2 cytoplasmic tail. Network hubs and interacting partners are co-expressed with Trop-2 in primary human tumours, supporting a role of this signalling network in cancer growth.

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INTRODUCTION

Trop-2 belongs to a unique family of transmembrane glycoproteins,[1–3] that has a regulatory role in cell–cell adhesion.[4,5] The Trop proteins have structural features that are not related to those of the four classical adhesion molecule families, that is, integrins, cadherins, selectins and Immunoglobulin-CAM.[6–8] The extracellular domains of the Trop proteins contain a globular portion with a GA733 type-I domain[9] and a thyroglobulin type-IA motif, which are required for Trop homo-multimerisation.[5] A cysteine-free region acts as a 'stem' to connect the globular moiety of the Trop proteins to their single hydrophobic transmembrane segments. The Trop proteins have a short (25–26 amino-acid-long) intracytoplasmic C-terminal tail, which appears to be devoid of enzymatic domains. Rather, it contains a HIKE-like phosphoinositide-binding motif, which is frequently present in signal transducers and which can act as a docking site for regulatory/effector molecules.[2,10,11]

We have shown that Trop-2 is overexpressed by most human cancers, as compared with their tissue of origin, and that Trop-2 quantitatively stimulates cancer growth *in vitro* and *in vivo*.[12] However, little is known about the regulation of expression of the *TROP2* gene. Hence, we investigated its regulatory network. *TROP2* expression was shown to depend on a highly connected network of transcription factors, which includes TP63/TP53L, ERG, GRHL1/Get-1 (grainyhead-like epithelial transactivator), HNF1A/TCF-1 (T-cell factor), SPI1/PU.1, WT1 (Wilms' tumour), GLIS2, autoimmune regulator (AIRE), forkhead box M1 (FOXM1) and FOXP3. *TROP2* upregulation was shown to subsequently drive the expression of cancer-growth-regulatory downstream effectors, that is, cyclic AMP-responsive-element binding protein (CREB), Jun, NF-κB, Rb, STAT1 and STAT3,[13–17] through the cyclin D1 and ERK (extracellular signal regulated kinase)/MEK (MAPK/ERK kinase) pathways. Signalling by the intact Trop-2 cytoplasmic tail was shown to be required for cell growth, as deletion of the Trop-2 cytoplasmic tail abolishes both modulation of NF-κB, cyclin D1 and pERK, and stimulation of growth. Trop-2 and main network partners appear to be concordantly upregulated in primary human tumours, supporting the *in-vivo* relevance of the Trop-2 signalling network for cancer growth.

RESULTS AND DISCUSSION

The Trop-2 signalling network

Upregulation of *TROP2* was shown to be necessary and sufficient to quantitatively stimulate tumour growth.[12] Upregulation of molecules involved in tumourigenesis is often a consequence of gene amplification (for example, *HER2/neu*).[18] Alternatively, inactivating mutations (for example, *TP53*) can drive compensatory overexpression.[19] However, we did not identify instances of mutations[12] or of amplification of the *TROP2* gene, which indicates that *TROP2* overexpression does not arise from structural alterations of the gene itself. Therefore, we sought to identify the network of transcription factors that can modulate the expression of *TROP2*. To capture actual *in-vivo* relevance, knockout models were analysed where ablation of specific genes was achieved (7 of 12 as gene replacement by homologous recombination) (Figure 1). DNA microarray analysis demonstrated that *TROP2* expression is modulated through the inactivation of TP63/TP53L (a TP53 family member), ERG (of the ETS family), GRHL1/Get-1, lymphoid enhancer factor/TCF-1/HNF1A, SPI1/PU.1, WT1, the Kruppel-like zinc finger transcription factor GLIS2, the autoimmunity regulator transcription factor AIRE, and the forkhead transcription factors FOXM1 and FOXP3 (Figure 1).

We then proceeded to determine whether these *TROP2*-modulating transcription factors can take part in a functionally relevant signalling module, and if this has a role in cancer. Ingenuity pathways analysis shows that the *TROP2*-modulating transcription factors can form a tightly interconnected *TROP2*-

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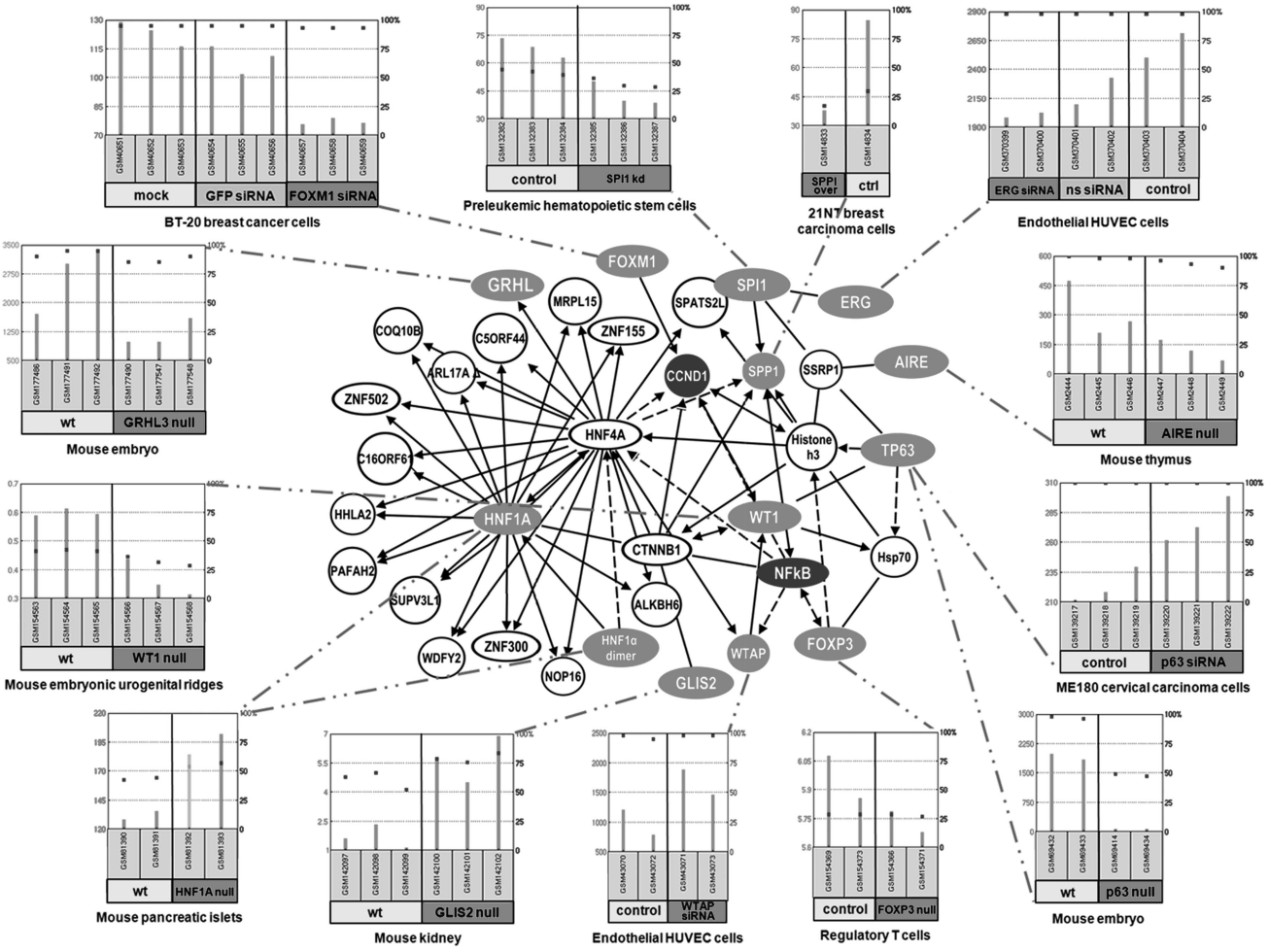

Figure 1. *TROP2* transcription network. Map of *TROP2*-driving transcription factors (centre). Proteins upstream and downstream of Trop-2 were examined using the Ingenuity Pathways Analysis software (version 8.8, Ingenuity Systems, www.ingenuity.com). All of the molecules were mapped onto the Ingenuity Knowledge Base as focus points. Networks of focus molecules were generated by maximising interconnectedness versus random distributions. Networks were ranked by a score based on a hypergeometric distribution and quantified as –log of right-tailed Fisher's exact test result. The transcription-factor network score was 34 (Fisher's exact test: 1×10^{-34}). The Trop-2-modulating proteins are in grey and connecting proteins are in white. Hubs shared between *TROP2* upstream/downstream transcription networks are in black. Bar graphs: vertical bars, value measurements of the GEO sample records (in arbitrary units; the record code is indicated in the grey boxes below); squares, rank order as percentile bins, which indicates where the expression of each gene falls with respect to all of the other genes on that array. Faded bars/squares correspond to absent Affymetrix detection call. Ranks are on a scale of 1–100% on the right *Y*-axes; the value scale is on the left *Y*-axes. Experimental groups are indicated for each set of replicate records. Cell/tissue sources are reported below each chart; regulatory T and preleukemic hematopoietic stem cells are of murine origin, ME180 cervical carcinoma, BT-20 and 21NT breast carcinoma and endothelial HUVEC cells are of human origin. A full colour version of this figure is available at the *Oncogene* journal online.

focused transcription-regulatory module. This module includes WT1-associated protein and osteopontin/SPP1, and it converges on six major hubs (that is, with $\geqslant 5$ connectors), with HNF4A as the most relevant one, with 138 edges (Figure 1). Strikingly, 20 of the 36 molecules in the network have roles in cancer and tissue development, and funnel signalling towards cyclin D1 and NF-κB as downstream effectors. TP63/TP53L, which is a homologue of the tumour suppressor TP53, drives the expression of genes that are involved in cell adhesion, proliferation and death.[20] Loss of endogenous p63 results in upregulation of genes associated with invasion, metastasis and epithelial–mesenchymal transition.[21] ERG is a transcription factor that regulates endothelial cell-adhesion molecules (for example, VE-cadherin and ICAM-2) and interleukin-8.[22] Mutations of GRHL1/Get-1 affect multiple genes that are linked to terminal differentiation, including structural proteins, lipid metabolising enzymes and cell-adhesion molecules.[23] Hypoxia inducible factor-1α modulates Wnt/β-catenin signalling in hypoxic embryonic stem cells through the enhancement of β-catenin activation and the expression of HNF1A/TCF-1.[24] HNF1A/TCF-1 is the largest hub in this *TROP2* network, and was shown to be essential for stem-cell growth. Constitutive transcription of TCF target genes caused by the loss of APC function is an early event in transformation of colonic epithelium.[25] Of note, TCF-1 acts as a feedback transcriptional repressor of β-catenin–TCF-4 target genes, and disruption of this negative feedback loop induces the formation of intestinal tumours, much like the loss of APC. SPI1/PU.1 has a crucial function in germinal progenitors. PU.1 knock-down leads to acute myeloid leukaemia in animal models. PU.1 is also involved in the development of breast cancer.[26] WT1 is a transcription factor that is critical for urogenital development and it has a complex role in tumorigenesis,[27] for example, through regulation of G2/M transition *via* stabilisation of the cyclin A2 messenger RNA.[28] GLIS2 is involved in kidney development, and loss of GLIS2 causes

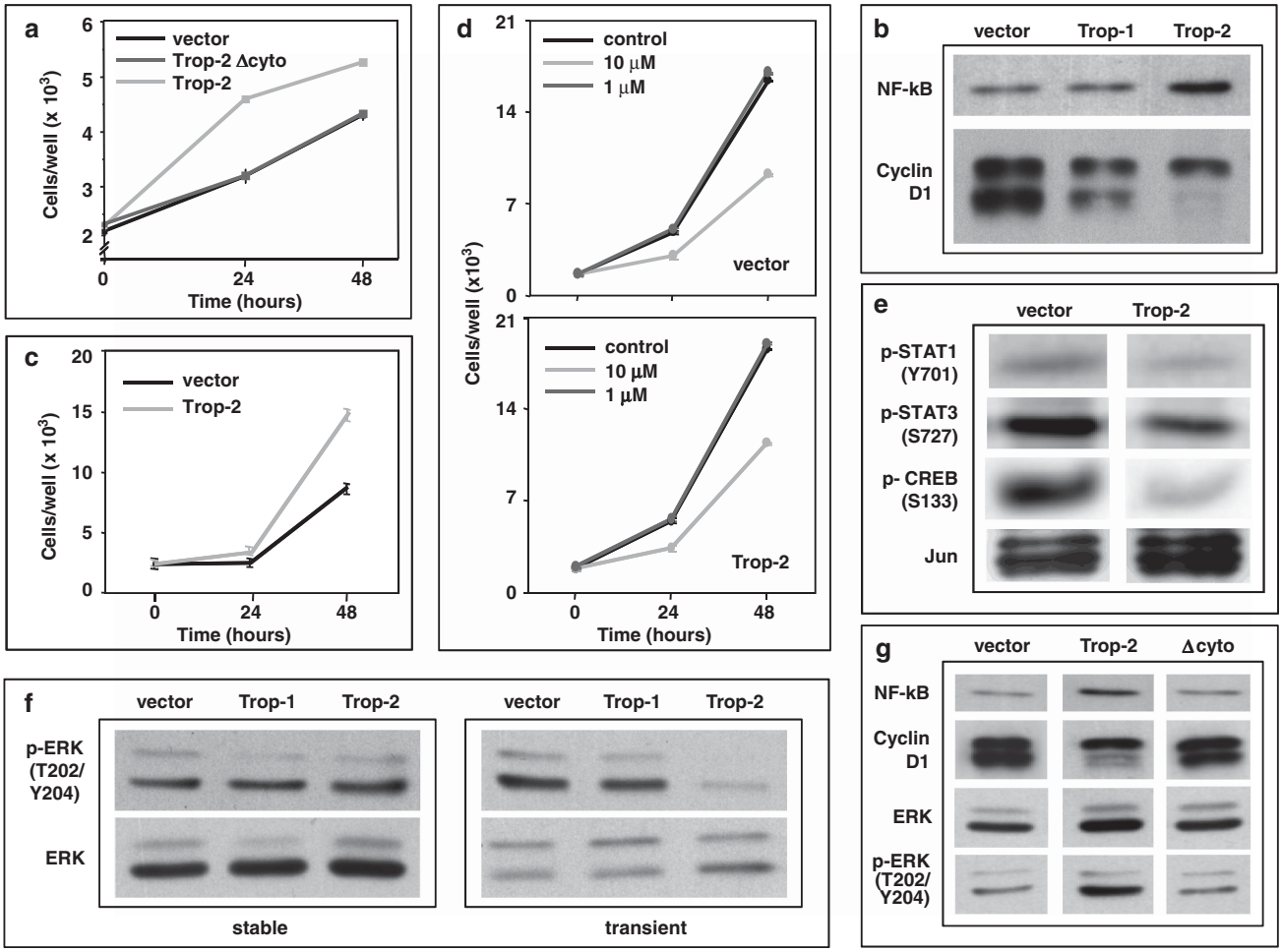

Figure 2. Trop-2 cytoplasmic tail-dependent cell-growth stimulation and signalling. The murine immortalised MTE 4–14 cells[49] were maintained in Dulbecco's modified Eagle medium, supplemented with 10% foetal bovine serum. Human wild-type *TROP2* (X77753) and the cytoplasmic domain mutants were obtained by polymerase chain reaction from the original full length *TROP2* clone[1] and inserted into the pΔEYFP expression vector. The human *TROP1* coding sequence was cloned in the pΔEYFP expression vector.[34] DNA transfection[50] was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Cell lysates were analysed by western blotting, as previously described.[2] Primary antibodies: monoclonal anti-cyclin-D1 (sc-246), goat polyclonal anti-NFκB p50 (sc-1190), anti-ERK-1 (sc-94-G) or anti-phospho-ERK-1/2 (Thr 202/Tyr 204) (sc-1698) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). CREB1 S133, Jun, STAT1 Y701, STAT3 S727 levels were determined by Kinexus Kinetworks. Signal intensity was quantified with NIH-image 1.62, using a Kodak grey-scale standards power curve (www.kodak.com) as reference. Cells were seeded at 3×10^3 cells/well in 96-well plates (five replica wells per data point). Cell numbers were quantified by staining with crystal violet (Sigma).[51] (**a**) Growth curves of MTE 4–14 cells transfected with vector, wild-type *TROP2* and cytoplasmic tail deletion *TROP2* mutants (Δcyto). Data are means ± s.d. (**b**) Western blot of Trop-associated cyclin D1 and NF-κB expression levels in MTE 4–14 cells transfected with vector alone, or as stable *TROP1* and *TROP2* transfectants. (**c**) Growth curves of MTE 4–14 cells transiently transfected with vector alone or with *TROP2*. Data are mean ± s.e.m. of the mean. (**d**) ERK pathway inhibition. Growth curves of MTE 4–14 cells transfected with vector alone (top) or with *TROP2* (bottom), and treated with 1 and 10 μM A6355 (Sigma) to inhibit ERK activity. Control cells were treated with vehicle alone. Data are means ± s.e.m. (**e**) Western blot of Trop-2-associated STAT1, STAT3, CREB and Jun levels and phosphorylation. MTE 4–14 cells were stably transfected with vector alone or *TROP2*. (**f**) Western blots of ERK absolute levels and phosphorylation in MTE 4–14 vector-alone transfectants, or in stable (left) or transient (right) *TROP1* and *TROP2* transfectants. (**g**) Western blot of Trop-2-tail-dependent NF-κB, cyclin D1 and ERK levels and phosphorylation. MTE 4–14 cells were stably transfected with vector alone, wild-type *TROP2* and cytoplasmic tail deletion *TROP2* mutants (Δcyto). A full colour version of this figure is available at the *Oncogene* journal online.

nephronophthisis through the induction of increased apoptosis and fibrosis.[29] Patients who express a defective form of the transcription factor AIRE develop a multiorgan autoimmune disease.[30] The FOXP3 transcription factor is associated with metastatic disease and poor prognosis in patients with breast cancer.[31] FOXM1 regulates G2-M transition, and its activity is stimulated by Ras and by p53 mutations, and is modulated by the PI3K-Akt pathway. FOXM1 is highly expressed in breast, hepatocellular and basal cell carcinomas, and is a marker of anaplastic thyroid carcinomas. Loss of FOXM1 leads to mitotic spindle defects, centrosome amplification and mitotic catastrophe.[32]

The Trop-2 downstream effectors

These findings led us to investigate the transcription-factor network downstream of Trop-2. High-throughput western blotting of MTE 4–14 transfectants revealed that Trop-2 overexpression drives upregulation of Jun (+56.2%, *versus* vector-transfected cells) and NF-κB (+111.5%), and loss of phosphorylation of CREB1 S133 (−81.1%), STAT1 Y701 (−17.4%), STAT3 S727 (−40.4%) (Figures 2b,e and g), and Rb S780 (−58.3%).

NF-κB and cyclin D1 appeared as major signalling effectors downstream of *TROP2*-modulating transcription factors (Figure 1), and were further investigated (Figures 2b and g). Supporting our network model, we show a *TROP2*-dependent modulation of

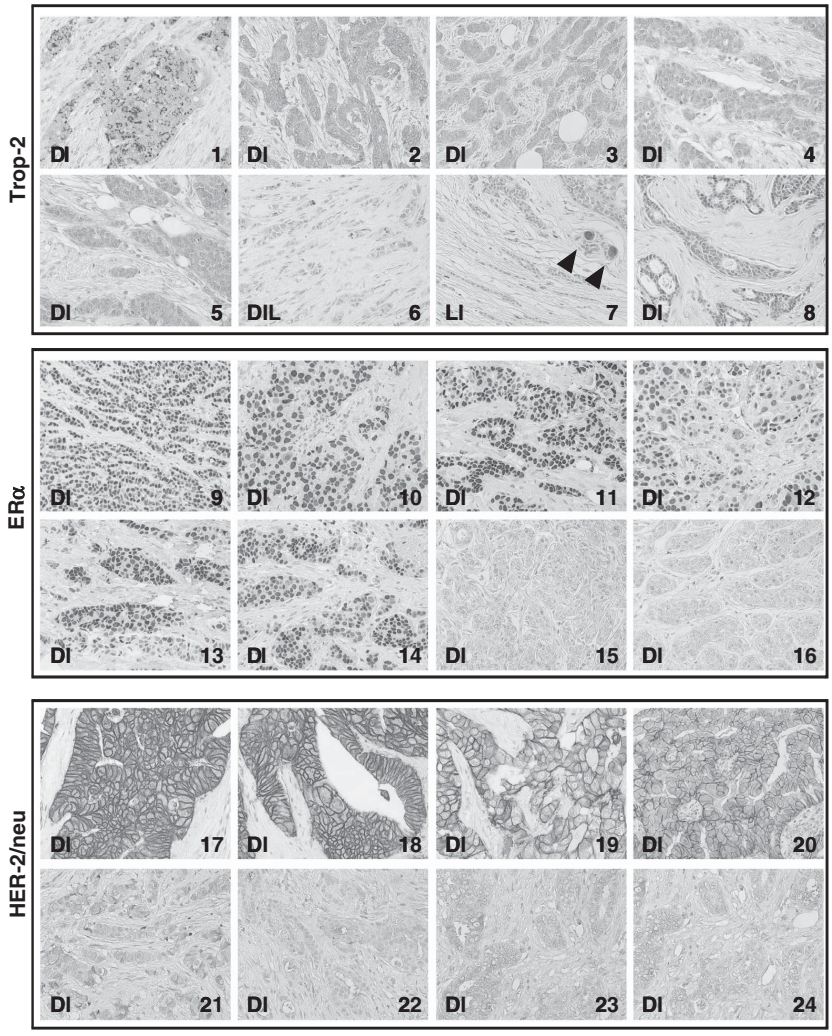

Figure 3. Trop-2, ERα and HER-2/neu expression in human breast cancer. Breast cancer samples analysed by immunohistochemistry. Sample tissue microarrays were assembled as previously described.[52] Endogenous peroxidase was blocked by exposure to 3% H_2O_2 for 5 min. Slides were treated with 0.3% bovine serum albumin in Tris-buffered saline at room temperature for 30 min to quench non-specific binding. The slides were then stained with the specific antibodies (Trop-2: 162–46.2 monoclonal;[44,53] ERα: MU368-UC monoclonal, Biogenex, Fremont, CA, USA; progesterone receptor: 1A6, Ventana, Tucson, AZ, USA; HER-2/neu: Hercep-Test (Dako, Glostrup, Denmark)[54] (Supplementary Table 1a). The bound antibodies were detected with goat antirabbit or antimouse horseradish peroxidase-labelled polymerised secondary antibodies (DAKO EnVision TM System). Peroxidase activity was visualised with diamminobenzidine (Dako). Slides were scored by two independent observers (MP, RL). The images are representative cases of ductal (DI) (1–5, 8–24), ducto-lobular (DLI) (6) and lobular (LI) (7) cancers. Arrowheads, calcified deposits (7). Expression levels were classified as described. (class 3): 1–5, 9–11; (class 2): 12–14, 19, 20; (class 1): 21; (low/negative): 6–8, 15, 16, 22–24; 1: granular positivity. All pictures were taken with a × 40 objective. A full colour version of this figure is available at the *Oncogene* journal online.

cyclin D1 and NF-κB (Figures 2b and g). We used the *TROP2* paralogue *TROP1*/Ep-CAM as a benchmark,[1,33] as *TROP1* also has cancer-driving capacity.[34] The absolute levels of NF-κB were increased up to 2.5-fold by Trop-2 overexpression, whereas they were insensitive to Trop-1 (Figures 2b and g). Cyclin D1 levels were reduced by two-to-four-fold by Trop-2, by one order of magnitude less by Trop-1 (Figures 2b and g). These findings indicate a major regulatory role of Trop-2 on NF-κB and cyclin D1, and that Trop-2 transduces signals differently from Trop-1. This is the first evidence that the two members of the Trop family have diversified roles in signalling for growth, which might help to explain both the phylogenetic history of this gene duplication and the differential roles the Trops have in normal and neoplastic cells, arguably upon selective pressure for function *in vivo*.

Cyclin D1 and NF-κB modulation downstream of Trop-2 reveals a seamless integration between signalling networks upstream and downstream of *TROP2*. As shown in Figures 2b and g, cyclin D1 appears as a double band in extracts from non-human cell lines. These bands have been shown to correspond to differentially phosphorylated forms of cyclin D1,[35] with faster-migrating forms having a shorter half-life. Trop-2 decreases cyclin D1 expression overall, but has a much stronger effect on faster-migrating molecules, suggesting preferential modulation of specifically phosphorylated cyclin D1 isoforms.

CREB upregulation correlates with tumour aggressiveness and survival of cancer patients.[36] Stimulation of CREB phosphorylation promotes the formation of a β-catenin–CREB transcriptional complex, which is then recruited to the cyclin D1 promoter to activate transcription.[37] Cyclin D1 interacts with STAT3 to regulate gene activation, for example, of the p21waf1 CDK inhibitor gene, and its binding to the p21waf1 promoter prevents recruitment of histone acetylases and RNA polymerase II, which leads to inhibition of p21waf1 transcription.[38] Of note, *TROP2* can form an RNA-oncogene by trans-splicing to *CYCLIN D1*, and expression

Table 1. Spearman's correlation analysis of Trop-2 expression and cell proliferation parameters

	Trop-2
Ki-67	
Rho	0.086
P	0.078
Cyclin D1	
Rho	0.435
P	<0.0001
Cyclin E	
Rho	0.358
P	<0.0001
ERα	
Rho	0.196
P	<0.0001
PgR	
Rho	−0.034
P	0.486
HER-2	
Rho	0.218
P	0.007
p27	
Rho	0.552
P	<0.0001
p16 (N)	
Rho	0.322
P	<0.0001
p16 (C)	
Rho	0.371
P	<0.0001

Abbreviations: ERα, oestrogen receptor α; (C), cytoplasmic staining; (N), nuclear staining; PgR, progesterone receptor; Rho, Spearman's correlation coefficient; *P*, two-tailed *P*-value. Significant correlations are highlighted (grey shading).

of the transforming messenger RNA chimera is regulated both through the regulation of the fusion event and through an altered stability of the spliced messenger RNA.[39] Steady-state amounts of *CYCLIN D1* messenger RNA are crucial to its transforming ability[39,40] and are critically regulated at the transcriptional level.[39,41]

Trop-2 signalling through the ERK/MAPK pathway

The Trop-2 cytoplasmic tail has conserved structural motifs[2,10,11] that might have roles in Trop-2-dependent signalling. Therefore, we generated a cytoplasmic tail-deletion mutant of Trop-2 (Figure 2a; Δcyto) and tested its growth-stimulatory and signalling capacity. Cytoplasmic tail ablation completely abolished Trop-2-dependent growth stimulation *in vitro* (Figure 2a), which indicates that tail-driven signalling is essential for Trop-2-dependent growth. We then analysed the expression of cyclin D1 and NF-κB, and the phosphorylation of ERK in transfectants with wild-type Trop-2 or with this tail-deletion mutant. All of the Trop-2-induced modulatory changes reverted to control levels upon deletion of the Trop-2 cytoplasmic tail (Figure 2g). Hence, an intact Trop-2 tail structure is required for Trop-2 signalling for growth through ERK, NF-κB and cyclin D1.

Cyclin D1 is downstream of ERK/MAPK, and Trop-2 has previously been shown to activate ERK in gene-reporter assays.[42] Hence, we went on to define the physiological relevance of the ERK/MAPK pathway in Trop-2 signalling (Figures 2d and f). *TROP2* induced growth of transiently transfected cells (Figure 2c), as did *TROP1*/Ep-CAM. In the same cells though, Trop-2 acutely inhibited ERK phosphorylation at activatory sites (Figure 2f, right): ERK-1 phosphorylation was essentially abolished, while that of ERK-2 was reduced by 10-fold. The absolute levels of ERK-1 remained essentially the same, while ERK-2 levels were induced by 20%. This was at variance with Trop-1, which reduced ERK-1 phosphorylation by 35%, while inducing a 20% increase in ERK-1 absolute levels. On the other hand, upregulation of the ERK-1 and ERK-2 absolute levels and of their activatory phosphorylation at T202/Y204 (up to three-fold) was seen after prolonged expression of Trop-2 (several days) (Figure 2f, left). Thus, acute versus prolonged expression of Trop-2 induces opposite changes in ERK phosphorylation, while the Trop-2 growth-stimulatory effects are retained, which suggests a signalling-network-dependent, feedback-mediated modulation of ERK by Trop-2, rather than an early activation step. Consistent with this, inhibition of ERK kinase activity (1–10 μM A6355) inhibits growth of Trop-2 transfectants to the same extent as that of control cells (Figure 2d). The ERK pathway was affected by the deletion of the Trop-2 cytoplasmic tail, and both absolute levels and phosphorylation at T202/Y204 of ERK-1 and ERK-2 reverted to control levels in the cytoplasmic tail deletion Trop-2 mutant (Figure 2g), although this appeared to be time-dependent in cell culture.

Trop-2 signalling in human tumours

Our findings have shown that Trop-2-driven signalling is essential for the growth of human cancer cells *in vitro* and in pre-clinical models.[12] Hence, we went on to assess the relevance of this signalling mechanism in primary human tumours. A prediction of our model is that key members of a Trop-2-dependent signalling module (cyclins/cyclin inhibitors, p53) would be concurrently expressed with Trop-2 in primary human neoplasias. Cluster analysis[43] led us also to predict concurrent expression with other breast cancer growth stimulators, like oestrogen receptor α (ERα) and HER-2/neu.

We examined a series of breast cancer cases for expression of Trop-2, as compared with the clinical and pathological characteristics of the tumours and with the levels of other progression, proliferation and differentiation markers (Figure 3; Supplementary Table 1a). Dichotomic high/low correlation versus Trop-2 levels first pinpointed a trend to a relationship with the expression of ERα ($P = 0.06$) and HER-2/neu ($P = 0.06$), when analysed as categorical variables (Supplementary Table 1a). Correlation between expression levels of Trop-2 and cell proliferation parameters as continuous variables was formally assessed *via* non-parametric Spearman correlation analysis (Table 1). Expression levels of Trop-2 were shown to strongly correlate with those of cyclin D1, cyclin E, the cyclin inhibitors p27 and p16, ERα and HER-2. Hence, specific proliferation and differentiation markers, which represent key components of the Trop-2 signalling network, are concurrently overexpressed with Trop-2 in primary tumours.

We then went on to analyse the expression profile of *TROP2*-linked transcription factors in human cancers (Figure 4). Two major patterns were apparent. The first one was a pattern of tumour-histotype-specific expression (FOXM1, ERG, TP63, WT1), with heterogeneous expression even within a specific tumour type. This suggested a corresponding heterogeneity of regulatory interactions with the *TROP2* gene, in particular in transcription factors overexpressing cancer samples. The second pattern was one of essentially ubiquitous expression (HNF1A, GRHL1, SPI1/PU.1, WT1, GLIS2, AIRE and FOXP3; HNF1A is shown as an example) (Figure 4; Supplementary Table 1b). This second pattern suggests a broad availability of a large fraction of *TROP2*-linked transcription factors for functional interactions in cancer cells.

The concordant upregulation of Trop-2 and of functionally linked cancer-driving molecules in human breast cancer supports functional relevance *in vivo*, and candidates the Trop-2 signalling network for novel diagnostic and therapeutic strategies.[44–48]

ABBREVIATIONS

AIRE, autoimmune regulator; CREB, cyclic AMP-responsive-element binding protein; FOXM1, forkhead box M1; Get, Grainyhead-like

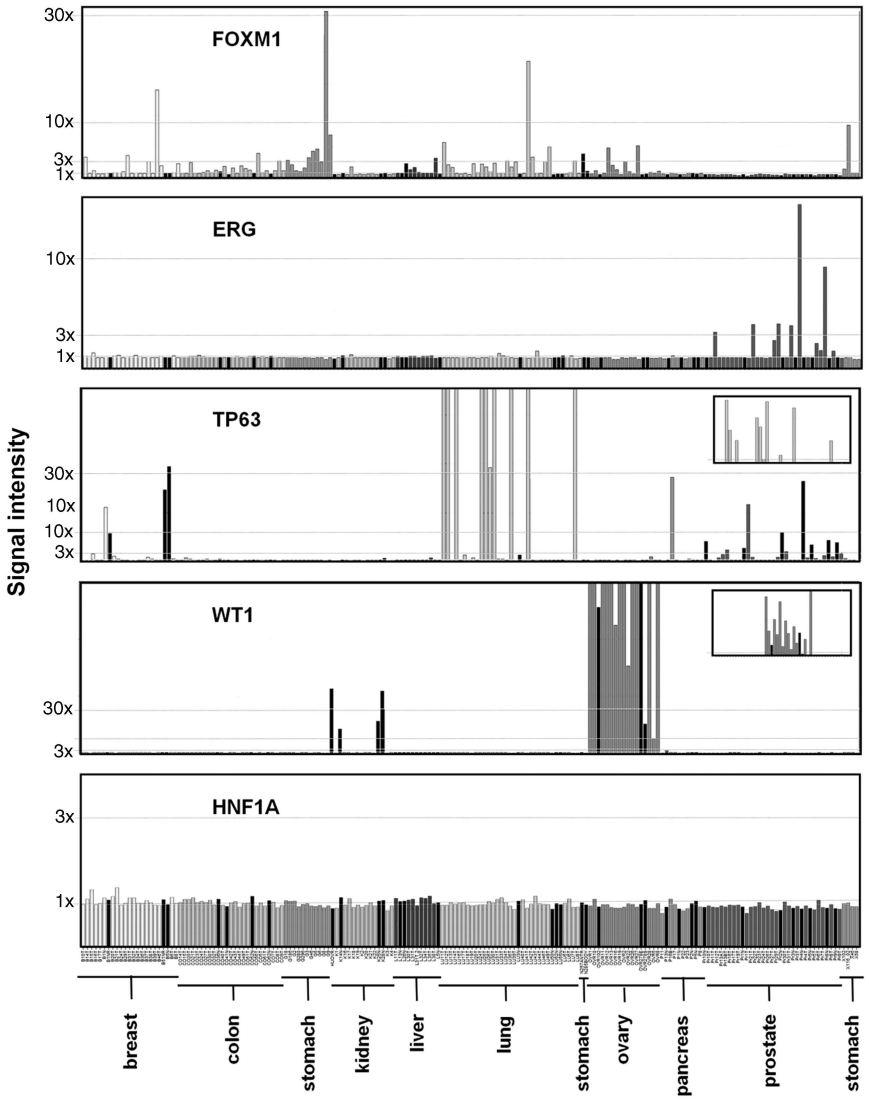

Figure 4. *TROP2*-linked transcription-factor expression in human normal and tumour tissues. Transcription-factor expression profiles from DNA microarray data sets (U95a GeneChip, Affymetrix, Santa Clara, CA, USA[55]) (biogps.org). Horizontal axes, cancer types (normal corresponding tissues are black bars; details on individual samples are in Supplementary Table 1b). Left vertical axes, fluorescence signal intensities (arbitrary units). Dotted horizontal lines, 1 ×, 3 × and 30 × median values. Insets: broken bars, showing out-of-scale expression of cancer samples. A full colour version of this figure is available at the *Oncogene* journal online.

epithelial transactivator; IPA, Ingenuity pathways analysis; TCF, T-cell factor; WTAP, Wilms' tumour-associated protein

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)

Supplementary Table S1a. Clinical and pathological features of the breast cancer patients case series.

	Trop-2 expression		
	low *number* (%)	high *number* (%)	P value
Age at diagnosis			
< 50	68 (25.5)	35 (22.4)	
> 50	199 (74.5)	121 (77.6)	0.56
Tumour size			
T1	182 (68.7)	94 (60.6)	
T2	83 (31.3)	61 (39.4)	0.11
Tumour grade			
I	33 (12.4)	19 (12.2)	
II-III	234 (87.6)	137 (87.8)	1.00
ERα			
Negative	72 (27.1)	30 (19.2)	
Positive	194 (72.9)	126 (80.8)	**0.06**
PgR			
Negative	127 (47.7)	67 (42.9)	
Positive	139 (52.3)	89 (57.1)	0.36
Ki-67			
Negative	144 (54.1)	85 (54.5)	
Positive	122 (45.9)	71 (45.5)	1.00
HER-2/neu			
Negative	241 (90.9)	130 (83.3)	
Positive	24 (9.1)	26 (16.7)	**0.03**

Abbreviations: ERα, oestrogen receptor α; PgR, progesterone receptor. Each IHC marker was analysed as categorical variable with validated cut-off values, as indicated in the text. Significant correlations are highlighted (grey shading).

Supplementary Table S1b. Clinical and pathological features of the primary tumours analysed for transcription factors expression.

ID	*histology*	*position*	*stage*	*lymph node status*	*markers*	*% tumour*	*tissue*
B1N	cancer						
B5N	normal						
B8T	cancer	left breast	T2	nd	PRunknown;ERunknown	75	
B10T	cancer	right breast	T4	positive	PRnegative;ERnegative	80	
B14T	cancer	right breast	T3	negative	PRunknown;ERunknown	80	
B16T	cancer	left breast	T1	negative	PRpositive;ERpositive	75	
B17T	cancer	left breast	T4	nd	PRunknown;ERunknown	85	
B1NR	normal						
B20T	cancer	right breast	T2	positive	PRpositive;ERpositive	80	
B51NR	normal						
B6T	cancer	right breast	T2	nd	PRunknown;ERunknown		
B15T	cancer	left breast	T1	negative	PRpositive;ERpositive	85	
B21T	cancer	left breast	T1	negative	PRnegative;ERnegative		breast
B29T	cancer	left breast	T2	negative	PRpositive;ERpositive		
B31T	cancer	left breast	T1	negative	PRnegative;ERnegative		
B32T	cancer	right breast	T3	positive	PRpositive;ERpositive		
B24T	cancer	left breast	T2	positive	PRpositive;ERpositive	80	
B46T	cancer	right breast	T3	positive	PRnegative;ERnegative	70	
B30T	cancer	right breast	T2	negative	PRnegative;ERnegative		
B34T	cancer	right breast	T2	positive	PRunknown;ERunknown	80	
B36T	cancer	left breast	T3	positive	PRunknown;ERunknown	85	
B37T	cancer	left breast	T2	negative	PRnegative;ERnegative	90	
B38T	cancer	left breast	T2	positive	PRpositive;ERpositive	80	
B39T	cancer	left breast	T1	negative	PRunknown;ERunknown	85	
B41T	cancer	right breast	T3	negative	PRnegative;ERnegative	85	
CO7T	cancer	right colon				70	
CO8T	cancer	sigmoid colon				90	
CO15T	cancer	caecum				50	
CO27T	cancer	right colon				75	
CO30T	cancer	sigmoid colon				75	
CO32T	cancer	caecum				85	
CO40T	cancer	right colon				70	
CO52N	normal (mucosa)						
CO62N	normal (muscularis)						
CO9T	cancer	rectum				60	
CO20T	cancer	right colon				70	
CO24T	cancer	sigmoid colon				80	
CO36N	normal (mucosa)						colon
CO42T	cancer	sigmoid colon				75	
CO14T	cancer	right colon				70	
CO21T	cancer	liver metastasis				90	
CO23T	cancer	liver metastasis				90	
CO41N	normal (mucosa)						
CO5T	cancer	non-rectal				80	
CO44T	cancer	transverse colon				90	
CO49T	cancer	sigmoid colon				85	
CO51T	cancer	sigmoid colon				85	
CO56T	cancer	right colon				85	
CO61T	cancer	non-rectal				80	
CO43T	cancer	sigmoid colon				80	
G2	cancer						
G3	cancer						
G5	cancer						
G6	cancer						
G8	cancer						
G9	cancer						
G18	cancer						
G46	cancer						
G186	cancer					70	stomach
G296	cancer					90	
G280	cancer					75	
N257ANTR	normal (antrum)						
N285BODY	normal (stomach body)						
X102	cancer					80	

X116_02	cancer				90	
X46	cancer				75	
X58	cancer				80	
K1	cancer (clear cell)	left	T1		90	
K2	cancer (clear cell)	left	T1		80	
K3	cancer (clear cell)	right	T2		85	
K4	cancer (clear cell)	right	T2		80	
K14N	normal					
K16	cancer (clear cell)	right	T1		90	
K17	cancer (clear cell)	left	T4M1		85	kidney
K19	cancer (clear cell)	left	T1N1		80	
K20	cancer (clear cell)	right	T1		85	
K22	cancer (clear cell)	right	T3A		85	
K18	cancer (clear cell)	right	T1		90	
K21	cancer (papillary)	left	T3A		90	
K25N	normal					
K28N	normal					
L11T	cancer		T3		85	
L13N	normal					
L30T	cancer		T3		80	
L5N	normal					
L31T	cancer		N1		70	
L32T	cancer		M		85	liver
L34T	cancer (recurrance)				75	
L35T	cancer		T3		90	
L38T	cancer				80	
L30T	cancer				85	
L31T	cancer					
L26N	normal					
LU11T	cancer (squamous cell)				50	
LU16T	cancer (adenocarcinoma)	right	T1		50	
LU7T	cancer (squamous cell)				50	
LU8T	cancer (adenocarcinoma)	right	T1		50	
LU12T	cancer (squamous cell)				65	
LU13T	cancer (squamous cell)				70	
LU5T	cancer (adenocsquamous)				50	
LU6T	cancer (adenocarcinoma)	right	T1		40	
LU14T	cancer (squamous cell)				50	
LU17T	cancer (adenocarcinoma)	right	T1		50	
LU50N	normal					
LU52N	normal					
LU18T	cancer (adenocarcinoma)	left	T1		70	
LU55N	normal					
LU3N	normal					
LU19T	cancer (squamous cell)				70	
LU20T	cancer (adenocarcinoma)	right	T2		70	lung
LU24T	cancer (squamous cell)				70	
LU25T	cancer (squamous cell)				70	
LU31T	cancer (adenocarcinoma)	right	T1		60	
LU34T	cancer (adenocarcinoma)	right	T1		70	
LU39T	cancer (adenocarcinoma)	left	T1		80	
LU40T	cancer (adenocarcinoma)	left	T1		50	
LU41T	cancer (squamous cell)				70	
LU44T	cancer (adenocarcinoma)	left	T1		70	
LU46T	cancer (neuroendocrine)				90	
LU49T	cancer (large cell undifferentiated)				90	
LU42T	cancer (small cell undifferentiated)				95	
LU30T	cancer (squamous cell)				65	
LU43T	cancer (small cell undifferentiated)				50	
LU26T	cancer (squamous cell)				65	
LU33T	cancer (adenocarcinoma)	left	T1		50	
LU36T	cancer (adenocarcinoma)				50	
OVR16	cancer (serous papillary)					
OVR19	cancer (serous papillary)					
OVR27	cancer (serous papillary)					
HUOVR	normal (commercial)					
OVR102	normal					
OVR28	cancer (serous papillary)					
OVR278E	normal (epithelial enriched)					
OVR2	cancer (serous papillary)					

OVR5	cancer (serous papillary)			ovary
OVR8	cancer (serous papillary)			
OVR11	cancer (serous papillary)			
OVR12	cancer (serous papillary)			
OVR13	cancer (serous papillary)			
OVR22	cancer (serous papillary)			
OVR26	cancer (serous papillary)			
OVR10				
OVR278S	normal (stromal enriched)			
OVR1	cancer (serous papillary)			
P1N	normal			pancreas
P2N	normal			
P5N	normal			
P8	cancer			
P11	cancer			
P17	cancer			
P22	cancer			
P13N	normal			
P16B	cancer			
P23	cancer			
Pr1T	cancer	T2AN0M0	75	prostate
Pr4T	cancer	T2bN0M0	80	
Pr5T	cancer	T2AN0M0	80	
Pr7T	cancer	T3AN0M0	60	
Pr1N	normal			
Pr5N	normal			
Pr6T	cancer	T2BN0M0	70	
Pr3N	normal			
Pr3T	cancer	T2BN0M0	85	
Pr4N	normal			
Pr2N	normal			
Pr8N	normal			
Pr8T	cancer	T3BN0M0	70	
Pr9N	normal			
Pr10N	normal			
Pr10T	cancer	T2AN0M0	85	
Pr9T	cancer	T2BN0M0	65	
Pr11T	cancer	T2AN0M0	80	
Pr12T	cancer	T3AN0M0	65	
Pr13AT	cancer	T3AN0M0	75	
Pr13BT	cancer	T3AN0M0	75	
Pr21T	cancer	TXNXMX	85	
Pr22T	cancer	T3AN0M0	75	
Pr30T	cancer	T3B0M0	80	
Pr23T	cancer	T3AN0M0	70	
Pr19T	cancer	T3N0M0	70	
Pr26T	cancer	T3ANXMX	70	
Pr31T	cancer	T2BN0M0	65	
Pr16T	cancer	T2BN0M0	75	
Pr17T	cancer	TXN1M0	90	
Pr24T	cancer	TXN0M0	85	
Pr27T	cancer	TXNXMX	65	
Pr29T	cancer	T2AN0M0	50	

Samples are grouped according to their tissue of origin. Tumours are colour coded as follows: yellow, breast ; green, colon; magenta, stomach; orange, kidney; blue, liver; cyan, lung; red, ovary; brown; pancreas; purple, prostate. Black highlights: normal tissues within each group.