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CLONING OF THE MURINE *TROP2* GENE: CONSERVATION OF A PIP_2-BINDING SEQUENCE IN THE CYTOPLASMIC DOMAIN OF TROP-2

Tarek EL SEWEDY[1], Mara FORNARO[1,2] and Saverio ALBERTI[1]*

[1]*Unit of Experimental Oncology, Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche Mario Negri—Consorzio Mario Negri Sud, Santa Maria Imbaro (Chieti), Italy*

Trop-2 is a novel calcium signal transducer expressed at high levels by most human carcinomas. To develop an animal model to study the function of this molecule *in vivo,* we have cloned the murine *Trop2* gene. Using human *TROP2* primers, we amplified by PCR a segment of murine *Trop2.* This was used as a probe to clone a full-length gene by hybridization of a genomic library. The cloned murine *Trop2* gene is functional, as indicated by sequencing and by expression after transfection. The murine Trop2 is 87.4% similar to its human homologue, with the highest conservation in the extracellular region between residues 86 and 157. Essentially all cysteines are conserved between the human and the murine genes, suggesting conservation of the Trop2 disulfide bridges and of its overall structure. Intriguingly, the cytoplasmic tail of Trop2 shows a highly conserved phosphatidylinositol 4,5-bisphosphate (PIP_2)-binding sequence, which overlaps with a protein kinase C phosphorylation site. Thus, we speculate that PIP_2 might regulate the phosphorylation state of Trop2 and play a role in its signal transduction. Murine Trop2 mRNA is detected in normal kidney, lung, ovary and testis, similarly to the human gene. Interestingly, the highest levels of expression are found in immortalized keratinocytes. Since Trop2 is undetectable in undifferentiated spindle cell carcinomas, this suggests a preferential expression at early stages of tumor progression. ***Int. J. Cancer*** **75:324–330, 1998.**
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The human Trop2/GA733-1/EGP-1 is a cell-surface glycoprotein expressed at high levels by the majority of human carcinomas but scarce in or absent from most normal tissues. The most relevant exceptions are multi-stratified epithelia and the placental trophoblast (Fornaro *et al.,* 1995; Lipinski *et al.,* 1981; Fradet *et al.,* 1984; Miotti *et al.,* 1987; Alberti *et al.,* 1992; Stein *et al.,* 1993). Following standard nomenclature rules, the human gene is called *TROP2* (Fornaro *et al.,* 1995), whereas the murine gene is indicated as *Trop2* (Morse, 1992). Trop2 is used for both the human and the murine proteins. Trop2 is phosphorylated by protein kinase C on serine 303 (Basu *et al.,* 1995) and thus was proposed to have a role as a signal transducer. Cross-linking Trop2 with antibodies causes a transient increase of intracellular calcium levels (data not shown), indicating that Trop2 is a cell-surface receptor that might recognize specific ligand(s) and transduce a calcium signal upon binding. The homologue Trop1/KSA/GA733-2 (Miotti *et al.,* 1987; Strnad *et al.,* 1989; Linnenbach *et al.,* 1993) has been suggested to be an adhesion molecule (Litvinov *et al.,* 1994), and a similar role might be played by Trop2. Different adhesion molecules, *e.g.,* integrins, can transduce calcium signals (Schwartz *et al.,* 1993) and regulate several responses linked to adhesion to a substrate, including cell growth (Assoian, 1997). A similar potential role of Trop2 would be consistent with the high expression of this molecule in tumor cells and with the finding that human cancer cells express an oncogenic chimeric mRNA between cyclin D1, a master regulator of the G_1-S transition in the cell cycle (Baldin *et al.,* 1993), and *TROP2* (data not shown).

Although the above findings suggest a potential role of Trop2 in tumor growth, *in vivo* evidence is missing. Thus, to develop an animal model to study the function of this molecule *in vivo,* we undertook cloning of the murine *Trop2* gene. A murine gene was considered necessary for these studies since the homologous human and murine Trop1 proteins (Miotti *et al.,* 1987; Strnad *et al.,* 1989; Linnenbach *et al.,* 1993; Bergsagel *et al.,* 1992) show 18% of non-conserved residues (Bergsagel *et al.,* 1992) and a similar divergence has to be expected for Trop2. Thus, the use of a murine gene would have avoided possible species-restricted Trop2/ligand(s) interactions. Determination of the murine *Trop2* sequence also was expected to define the most evolutionarily conserved regions of Trop2. These are likely to correspond to the most functionally relevant regions of this molecule and would be potential targets for functional and mutagenetic studies. Finally, the use of a murine *Trop2* probe would allow definition of its expression pattern in rodents and comparison with the human gene, as a further test of functional conservation.

We describe the cloning of the murine *Trop2* gene. We show that the structure and pattern of expression of Trop2 are conserved across species and identify conserved regions that are expected to play a crucial role in the function of this molecule. In particular, we show that the cytoplasmic tail of Trop2 has a highly conserved phosphatidylinositol 4,5-bis phosphate (PIP_2)-binding sequence (Yu *et al.,* 1992; Pitcher *et al.,* 1996), suggesting a PIP_2-regulated signaling of Trop2.

MATERIALS AND METHODS

Cells

The murine C5N immortalized keratinocytes and the A5 and Carc-B spindle cell carcinomas (Diaz-Guerra *et al.,* 1992; Burns *et al.,* 1991) were kind gifts of Drs. G. Portella (Naples, Italy) and A. Balmain (Glasgow, UK), respectively. C5N, A5, Carc-B, L, F9, F9-C1 (Strickland and Mahdavi, 1978), 3LL lung carcinoma cells and the mouse fibroblast L cells were maintained in DMEM (GIBCO, Grand Island, NY) supplemented with glutamine, antibiotics and 10% FCS (GIBCO).

PCR

Oligonucleotide primers were synthesized by Duotech (Milano, Italy). Twenty-four different combinations of 20-bp-long human *TROP2* non-degenerate primers were tested for amplification of murine *Trop2* by PCR. Thirty cycles of denaturation at 94°C for 1 min, annealing at temperatures between 45° and 55°C for 1 min and 1 min synthesis at 72°C were performed using a thermocycler (Perkin-Elmer Cetus, Branchburg, NJ). Murine genomic DNA was used as template since *Trop2* was expected to be without introns, like its human counterpart (Fornaro *et al.,* 1995). One pair of these primers (F3, R3) (Table I) amplified a 166 bp band of murine *Trop2.* Four degenerate oligonucleotide primers, F1, F2, R1 and R2 (Table I), were subsequently designed within 2 regions highly conserved between Trop1 and Trop2 (Fornaro *et al.,* 1995). These

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[2]Present address: Department of Pathology, School of Medicine, Yale University, New Haven, CT, USA.

*Correspondence to: Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro (Chieti), Italy. Fax: (39) 872 578.240. E-mail: alberti@cmns.mnegri.it

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were used in a nested PCR amplification. PCR products were analyzed by Southern blotting, using as probes the corresponding regions of the human *TROP2* gene. Hybridizing bands were purified, subcloned into pBluescript SK^+ (Stratagene, La Jolla, CA) and sequenced.

Genomic cloning

Several phage, cosmid or YAC mouse genomic libraries were screened by PCR or by hybridization, using as probes the human *TROP2* or the murine *Trop2* fragments amplified by PCR. A successful screening was performed on a murine genomic 129SV lambda FixII phage library (kindly donated by Dr. M. Davis, Stanford, CA), using as a probe the F2-R2 murine *Trop2* PCR fragment (Table I, Fig. 1). Filters were hybridized at high stringency (43°C, 5× SSPE and 50% formamide) and washed at 55°C in 0.1× SSPE (Sambrook *et al.,* 1989). One positive clone was isolated and purified by 2 successive rounds of screening. The *Trop2* phage DNA was analyzed by Southern blot using various probes scattered along the human *TROP2* open reading frame (ORF). A 2.1 kb Xba I segment that contained the entire murine *Trop2* ORF was isolated and sequenced.

DNA sequencing

DNA sequencing was performed following the method of Sanger (Sambrook *et al.,* 1989) using T7 Sequenase kits from USB (Cleveland, OH).

Southern and Northern blot analyses

Total RNA was extracted from murine cell lines. Poly-A^+ mRNA was extracted from fresh C57BL/6 mouse organs snap-frozen in liquid nitrogen (Sambrook *et al.,* 1989). Genomic DNA from the different sources was also extracted (Sambrook *et al.,* 1989; Alberti and Fornaro, 1990). Restriction enzymes were purchased from New England Biolabs (Beverly, MA) or Boehringer-Mannheim (Mannheim, Germany). Southern and Northern hybridizations were performed at high stringency (Sambrook *et al.,* 1989). Ten micrograms of DNA, 20 µg of total RNA or 1.5 µg of poly-A^+ RNA were loaded per lane. Reproducible DNA loading, transfer and hybridization were verified by the comparable signal intensity among different cellular sources of DNA (see below).

Production of recombinant murine Trop2 in Escherichia coli

A recombinant murine Trop2 was synthesized. Briefly, the F2-R2 *Trop2* segment was subcloned in the galactose-inducible pQE-10 vector (Qiagen, Chatsworth, CA). The correctness of the resulting ORF was confirmed by DNA sequencing. Synthesis of the recombinant murine Trop2 was induced by 0.2 mM IPTG for 5 hr. The 6 histidine-tagged recombinant murine Trop2 was purified by affinity chromatography on a chelated Ni^{2+} column (Qiagen) following the manufacturer's instructions. The purity of the recombinant Trop2 protein was verified by PAGE.

Anti-murine Trop2 anti-sera

Rabbit anti-mouse Trop2 anti-sera were produced as described (Ausubel *et al.,* 1959). Briefly, the recombinant murine Trop2 protein was cross-linked to keyhole limpet hemocyanin (KLH) (Sigma, St. Louis, MO) with glutaraldehyde following standard procedures (Ausubel *et al.,* 1959). The murine Trop2/KLH was emulsified in complete Freund's adjuvant and injected s.c. in rabbits (500 µg per animal). Subsequent immunizations in incomplete Freund's adjuvant were performed at 4-week intervals. Procedures involving animals and their care were conducted in conformity with the institutional guidelines and with national and international laws and guidelines.

TABLE I – PRIMERS USED IN THE PCR AMPLIFICATION OF THE MOUSE *TROP2*

Primer	Sequence
F1	AGCTCTAGATCSWGRACAAYGATGGNCT[1]
F2	CGATCTAGABTAYGAYCCYGACTGCGA
F3	GTCTGAGTGGTTGAAGGCGC
R1	CCAAAGCTTCCACCACRATGACRGCGA
R2	GACAAGCTTGABVCCGGCBGTGAGGC
R3	GCGCCAGTGCAACCAGACGT

[1]Degenerate bases are indicated following the IUB-GCG code (Devereux *et al.,* 1984): S, C or G; W, or T; R, A or G; Y, C or T; B, C or G or T; V, A or C or G; Xba I or Hind III restriction sites are underlined.

Western blot analysis

Western blots were performed as described (Ausubel *et al.,* 1959). Briefly, cell membrane preparations from 10^7 murine *Trop2* transfected or control cells were analyzed by SDS-PAGE and transferred to nitrocellulose filters. Filters were pre-hybridized in 5% milk for 18 hr and hybridized in the same solution with anti-murine Trop2 anti-serum for 1 hr at room temperature. Filters were washed for 20 min and further incubated with goat anti-rabbit peroxidase (Calbiochem, La Jolla, CA). After washing, antibody binding was revealed by enhanced chemiluminescence (ECL; Amersham, Aylesbury, UK).

DNA transfection

Murine *Trop2* was subcloned in the mammalian expression plasmid pBJI-neo (Lin *et al.,* 1990; kindly supplied by Dr. M. Davis). DNA transfection was performed by calcium phosphate co-precipitation (Alberti and Herzenberg, 1988). Transfected cells were selected in medium containing 500 µg/ml geneticine.

Computer sequence analysis

DNA sequences were analyzed using Genetics Computer Group programs (Devereux *et al.,* 1984). Peptide diagonal analysis was performed with the COMPARE and DOTPLOT programs. Multi-sequence conservation analyses between members of the Trop family were performed with the LINEUP, PILEUP and BOX-SHADE programs.

RESULTS

PCR amplification of murine Trop2

Low-stringency hybridization of murine genomic libraries with human *TROP2* led to the isolation of several spuriously hybridizing clones (data not shown). Thus, we searched for a murine *Trop2* probe to be used in high-stringency library screenings. We amplified by PCR segments of murine *Trop2* and verified their identity by sequencing. A pair of human primers (F3, R3; Table I) amplified a band of 166 bp corresponding to the mouse coding region between residues 110 and 165 (Fig. 1). A 553 bp murine *Trop2* fragment was amplified using 4 degenerate oligonucleotides, F1, F2, R1, R2 (Table I, Fig. 1), in a nested PCR. After sequencing, the 553 bp band was used as a probe in library screening.

Cloning of genomic murine Trop2

After an initial failure to isolate by hybridization murine *Trop2* from several genomic libraries (Super-CosI cosmids [Stratagene], Charon-4 and RIII phages, and YACs [Genome Systems, St. Louis, MO]), we screened by PCR a 129SV genomic library in lambda FIX II and showed that it contained a murine *Trop2*. Five hundred thousand plaques from this library were plated, transferred to nitrocellulose filters and hybridized with the murine F2-R2 segment at high stringency. After 3 rounds of screening, we isolated one murine *Trop2* clone. High-stringency Southern blots identified a single 2.1 band that contained the entire coding region, as confirmed by sequencing (Fig. 1).

Sequence analysis

The murine *Trop2* gene is intronless, like its human counterpart (Fornaro *et al.,* 1995), supporting the hypothesis that *TROP2* derives from a retro-position of *TROP1* in the genome (Linnenbach *et al.,* 1993). The murine *Trop2* ORF encodes a protein of 317 amino acids that is 79.2% identical and 87.4% similar to human Trop2. The hydrophobic plot of murine Trop2 corresponds to that

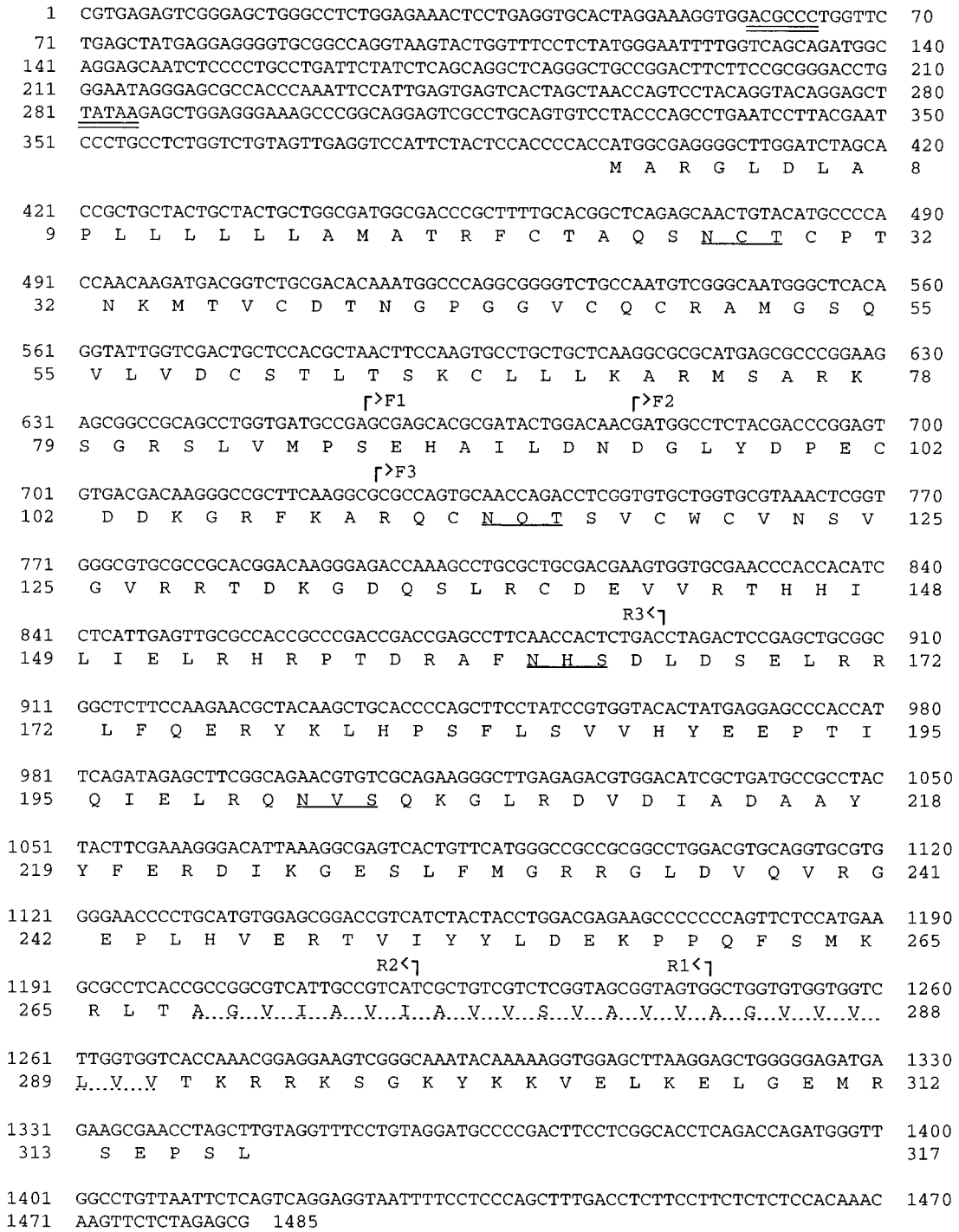

FIGURE 1 – Nucleotide and amino acid sequence of the cloned murine *Trop2* gene (accession number Y08830). Consensus TATA box and SP1-binding sites are doubly underlined. Potential glycosylation sites are underlined. The position of the primers used for PCR and the direction of amplification are indicated by arrows. The transmembrane region is indicated by a dotted underline. Residue numbers are shown on the side.

of human Trop2 and predicts a type I transmembrane protein with a hydrophobic leader peptide, devoid of the poly-proline stretch present in the human molecule (Fig. 2), and one transmembrane domain (Malthiery and Lissitzky, 1987). Comparison of the Trop1 and Trop2 polypeptide sequences identifies 3 regions that are conserved in all Trop molecules and that appear to characterize structurally the Trop family (Fig. 3*a*). Two of these regions are extracellular, while the third includes the transmembrane and cytoplasmic regions. Like all other Trop molecules (Linnenbach *et al.*, 1993; Bergsagel *et al.*, 1992), the murine Trop-2 cysteine-rich domain contains an EGF and a thyroglobulin repeat (Malthiery and Lissitzky, 1987) and 12 of its 13 extracellular cysteines are conserved with the human Trop2, indicating a similar folding and 3-dimensional structure. Remarkably, the only additional cysteine is in the leader sequence, which is expected to be cleaved from the mature protein. The highest conservation between human and

murine Trop2 is in the extracellular segment between residues 86 and 157 (Figs. 2, 3*b*).

The region of highest conservation in the cytoplasmic domain encompasses a sequence that is highly homologous to PIP_2-binding sites of gelsolin (Yu *et al.,* 1992) and of the G protein-coupled receptor kinase 4 (GRK4) (Pitcher *et al.,* 1996) (Fig. 4). Intriguingly, this PIP_2-binding site is not present in Trop1 molecules. A serine phosphorylation site (Basu *et al.,* 1995) and a potential tyrosine phosphorylation site (Cooper *et al.,* 1984) also are conserved in the human and murine Trop2 cytoplasmic regions (Figs. 1, 2).

Tissue and cell line expression of murine Trop2

We measured the mRNA levels of murine *Trop2* in several fresh tissues and cell lines. The murine *Trop2* 1.8 kb mRNA transcript was detected in kidney, lung, ovary and testis (Fig. 5*a*). Interestingly, quite high levels of expression of murine *Trop2* were detected in a murine immortalized keratinocyte C5N cell line,

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mTrop-2     1 MARGLDLA.......PLLLLLLLAMATRFCTAQSNCTCPTNKMTVCDTNGPGGVCQCRAMGSQVLVD
hTrop-2     1 MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVCSPDGPGGRCQCRALGSGMAVD
hTrop-1     1 MAPPQVLA........FGLLLAAATATFAAAQEECVCENYKLAVNCFVNNNRQCQCTSVGAQNTVI
mTrop-1     1 MAGPQALA........FGLLLAVVTATLAAAQRDCVCDNYKLATSCSLNEYGECQCTSYGTQNTVI

mTrop-2    60 CSTLTSKCLLLKARMSARKSGRSLVMPSEHALLDNDGLYDPECDDKGRFKARQCNQTSVCWCVNS
hTrop-2    66 CSTLTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCWCVNS
hTrop-1    59 CSKLAAKCLVMKAEMNGSKLGRRA..KPEGALQNNDGLYDPDCDESGLFKAKQCNGTSTCWCVNT
mTrop-1    59 CSKLASKCLAMKAEMTHSKSGRRI..KPEG.IQNNDGLYDPDCDEQGLFKAKQCNGTATCWCVNT

mTrop-2   125 VGVRRTDKGDQSLRCDEVVRTHHILIELRHRPTDRAFNHSDLDSELRRLFQERYKLHPSFLSVVH
hTrop-2   131 VGVRRTDKGDLSLRCDELVRTHHILIDLRHRPTAGAFNHSDLDAELRRLFRERYRLHPKFVAAVH
hTrop-1   122 AGVRRTDK.DTEITCSERVRTYWIIIELKHKAREKPYDSKSLRTALQKEITTRYQLDPKFITSIL
mTrop-1   121 AGVRRTDK.DTEITCSERVRTYWIIIELKHKERESPYDHQSLQTALQEAFTSRYKLNQKFIKNIM

mTrop-2   190 YEEPTIQIELRQNVSQKGLRDVDIADAAYYFERDIKGESLFMGRRGLDVQVRGEPLHVE..RTVI
hTrop-2   196 YEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKGESLFQGRGGLDLRVRGEPLQVE..RTLI
hTrop-1   186 YENNVITIDLVQNSSQKTQNDVDIADVAYYFEKDVKGESLFH.SKKMDLTVNGEQLDLDPGQTLI
mTrop-1   185 YENNVITIDLMQNSSQKTQDDVDIADVAYYFEKDVKGESLFHSSKSMDLRVNGEPLDLDPGQTLI

mTrop-2   253 YYLDEKPPQFSMKRLTAGVIAVIAVVSVAVVAGVVVLVVTKRRKSGKYKKVELKELGEMRSEPSL
hTrop-2   259 YYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGMAVLVITNRRKSGKYKKVEIKELGELRKEPSL
hTrop-1   250 YYVDEKAPEFSMQGLKAGVIAVIVVVVMAVVAGIVVLVISRKKRMAKYEKAEIKEMGEMHRELNA
mTrop-1   250 YYVDEKAPEFSMQGLTAGIIAVIVVVSLAVIAGIVVLVISTRKKSAKYEKAEIKEMGEIHRELNA
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FIGURE 2 – Multiple peptide sequence alignment of the different members of the Trop family. Murine (m) Trop2, human (h) Trop-2 (X77753), hTrop1 (M32306), mTrop1 (M76124). Identical or similar amino acids shared by 3 or more sequences are shaded in black or gray, respectively. Residue numbers are shown on the side.

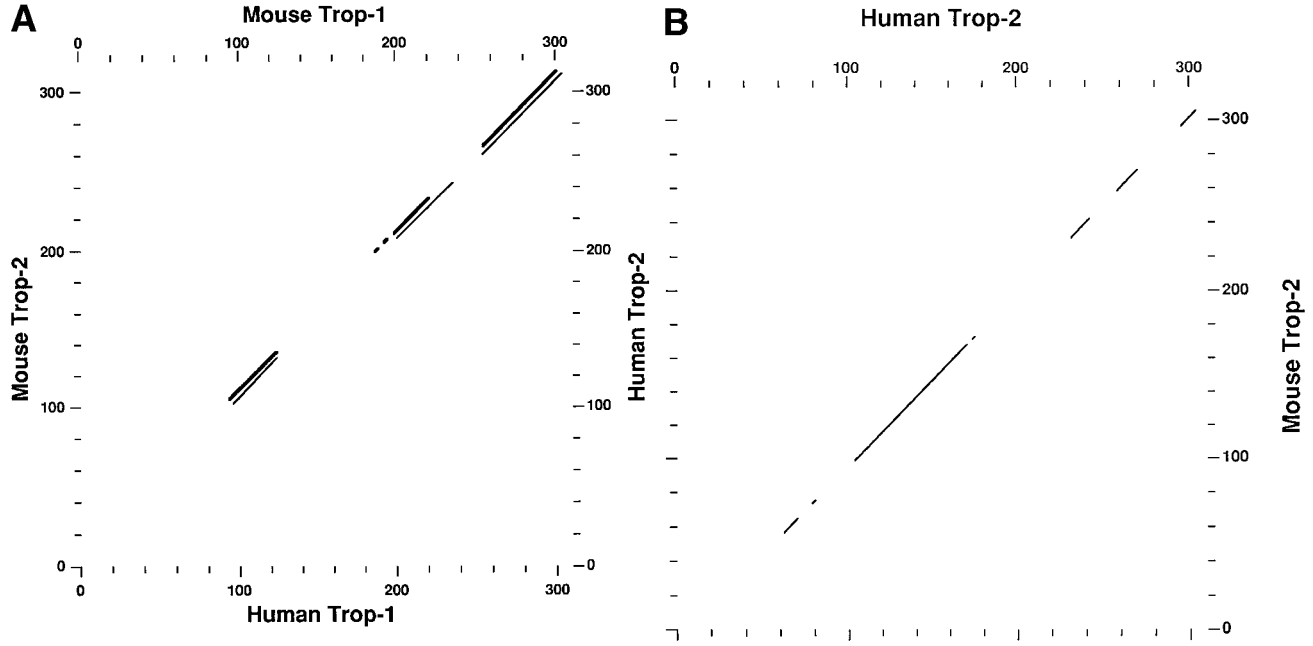

FIGURE 3 – Conserved regions in the Trop protein family. *(a)* Compound dot-plot comparison of Trop2 *vs*. Trop1 in humans or mice. Thick lines represent regions of homology between human molecules, while thin lines indicate regions of homology between murine molecules. This analysis was performed using a window of 30 residues and a stringency of 27. *(b)* Dot-plot comparison between murine and human Trop2 peptide sequences. The highest conservation is observed in one extracellular domain. The comparison was done using a window of 30 residues and a stringency of 40 (Devereux *et al.,* 1984).

hTrop-2	302	K	S	G	.	K	Y	K	K	308
mTrop-2	296	K	S	G	.	K	Y	K	K	302
GRK4	22	K	S	G	.	R	S	K	K	29
Gelsolin	162	K	S	G	L	K	Y	K	K	169

FIGURE 4 – The PIP_2-binding motif in Trop2, GRK4, gamma splice variant (U33055) and gelsolin (P06396). The dot indicates a 1 amino acid gap.

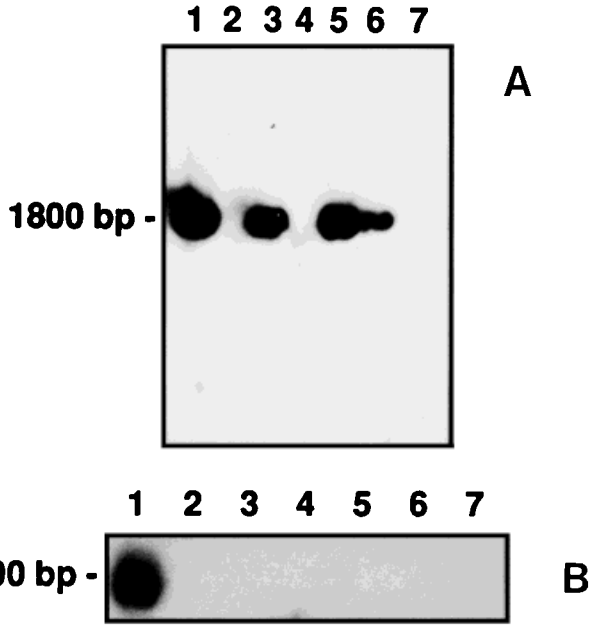

FIGURE 5 – Expression of the murine *Trop2* gene. *(a)* Northern blot of fresh murine tissues. Lane 1, kidney; lane 2, heart; lane 3, lung; lane 4, spleen; lane 5, ovary; lane 6, testis; lane 7, liver. *(b)* Northern blot of murine cell lines. Lane 1, C5N; lane 2, A5; lane 3, Carc-B; lane 4, F9; lane 5, F9C1; lane 6, L cells; lane 7, 3LL. Filters were hybridized with an F2-R2 murine *Trop2* probe.

whereas all spindle cell carcinomas tested lacked detectable *Trop2* expression (Fig. 5*b*).

Expressibility of cloned murine Trop2

Stable transfectants for murine *Trop2* were produced in murine L cells, and expression was confirmed by Northern blot (data not shown). Western blot analysis of plasma membrane preparations from these transfectants specifically identifies a 45 kDa protein (Fig. 6), proving that our murine *Trop2* clone is functional and can be expressed efficiently after transfection. The molecular weight of the transfected mouse Trop2 is about 10 kDa larger than predicted from the amino acid sequence. This probably is caused by glycosylation of one or more of the 4 potential glycosylation sites (Devereux *et al.*, 1984) (Fig. 1), as previously demonstrated for the human molecule (Alberti *et al.*, 1992).

Murine Trop2 *is a single-copy gene*

Southern blot analysis of murine genomic DNA digested with XbaI and hybridized at high stringency with the coding region of the murine *Trop2* reveals a single 2.1 kb band (Fig. 7), as predicted by the restriction map of the *Trop2* clone. Cutting genomic DNA with other restriction enzymes consistently produces only one or a few murine *Trop2* bands, which show an overall intensity comparable to other single-copy genes hybridized to probes of similar

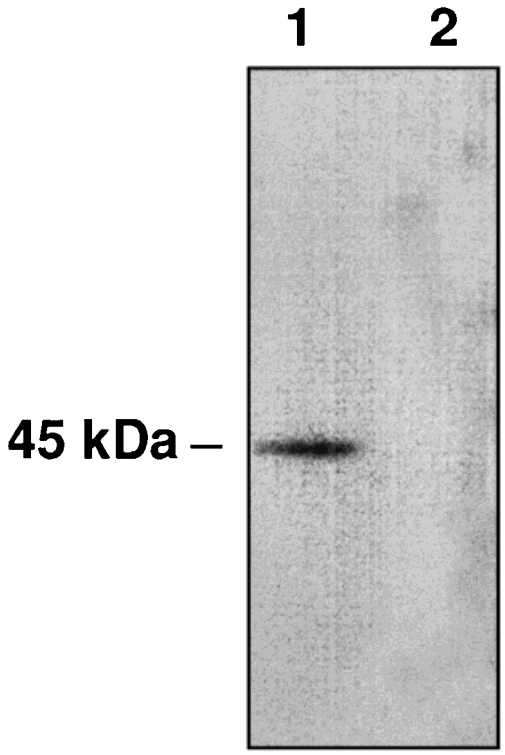

FIGURE 6 – Expressibility of the murine *Trop2* gene. Western blot analysis of murine *Trop2* transfectants. Lane 1, murine *Trop2*-transfected L cells; lane 2, L cells transfected with the selectable marker alone.

length (data not shown). The comparable signal intensity among different cellular sources of DNA excludes also the occurrence of gene amplification (Alberti *et al.*, 1994). Taken together, these findings indicate that *Trop2* is a single-copy gene in mice.

DISCUSSION

Human Trop2/GA733-1/EGP-1 is a cell-surface glycoprotein (Alberti *et al.*, 1992) expressed at high levels by the majority of human carcinomas (Lipinski *et al.*, 1981; Miotti *et al.*, 1987; Stein *et al.*, 1993). Phosphorylation of its cytoplasmic tail by protein kinase C (Basu *et al.*, 1995) and the intracellular calcium signaling caused by cross-linking with monoclonal antibodies (data not shown) indicate that Trop2 is a novel cell-surface signal transducer. These findings together with the high expression of this molecule in tumor cells suggest a potential role of Trop2 in tumor development.

To study the function of Trop2 *in vivo* in an animal model, we have cloned the murine *Trop2* gene. Two sets of primers corresponding to highly conserved regions in Trop molecules were used in a nested PCR and amplified a 553 bp murine *Trop2* segment. This was used to screen several phage and cosmid mouse genomic libraries by hybridization, leading to isolation of a full-length genomic *Trop2* from a 129SV murine library. *Trop2* is a single-copy gene in mice, as was previously shown in humans (Fornaro *et al.*, 1995). The cloned murine *Trop2* is a functional gene, as shown by sequencing and by expression of the murine Trop2 protein in transfected L cells. This genomic *Trop2* clone is syngenic with the murine ES embryonal carcinoma cells used in gene-targeting experiments to obtain knock-out mice (Deng and Capecchi, 1992); thus, it is suitable for *Trop2* gene-ablation experiments.

Murine *Trop2* encodes a type I transmembrane protein of 317 amino acids. The position and number of the extracellular cysteines of murine and human Trop2 are highly conserved, suggesting

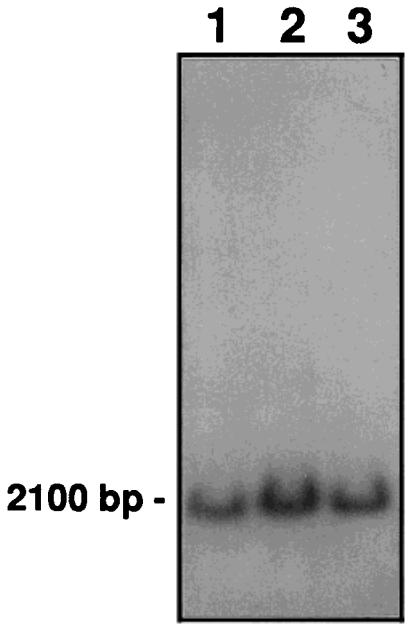

FIGURE 7 – Murine *Trop2* is a single-copy gene. Southern blot analysis of murine genomic DNA isolated from: lane 1, mouse tail; lane 2, F9 teratocarcinoma cell line; lane 3, L fibrosarcoma cell line. DNA was digested with Xba I.

conservation of disulfide bonds, of overall folding and of 3-dimensional structure. Sequence homology between murine and human Trop2 is particularly high in one extracellular domain, suggesting a critical function of this region of the molecule, *e.g.*, in the binding to Trop2 ligand(s). Intriguingly, the cytoplasmic tail of human and murine Trop2 contains a PIP_2-binding consensus sequence that is almost identical to a PIP_2-binding site found in gelsolin (Yu *et al.*, 1992) or GRK4 (Pitcher *et al.*, 1996) (Fig. 4). Gelsolin is an actin filament-severing and -capping protein (Yu *et al.*, 1992), and GRKs are cytoplasmic kinases that phosphorylate G protein-coupled receptors (Pitcher *et al.*, 1996). PIP_2 can regulate the binding of gelsolin and GRK4 to other cytoplasmic molecules (Yu *et al.*, 1992; Lambrechts *et al.*, 1997) or to the plasma membrane (Pitcher *et al.*, 1996). Thus, it might play a similar role for Trop2. Quite interestingly, the PIP_2-binding sequence of Trop2 encompasses a serine that can be phosphorylated by protein kinase C (Basu *et al.*, 1995). Thus, PIP_2 might regulate protein kinase C-mediated phosphorylation of Trop2 (Basu *et al.*, 1995). Consistently, a Trop2-like PIP_2-binding site in neurogranin can be phosphorylated by protein kinase C and the binding of PIP_2 increases the efficiency of phosphorylation (Lu and Chen, 1997).

We have shown that murine Trop-2 is transcribed in kidney, lung, ovary and testis, quite similarly to its human counterpart (Fornaro *et al.*, 1995; Lipinski *et al.*, 1981; Fradet *et al.*, 1984; Miotti *et al.*, 1987; Alberti *et al.*, 1992; Stein *et al.*, 1993). High levels of expression have been found in immortalized keratinocytes, whereas spindle cell carcinomas, *i.e.*, highly malignant cells at an advanced stage of tumor progression (Diaz-Guerra *et al.*, 1992; Burns *et al.*, 1991), do not express Trop2. These preliminary findings suggest that Trop2 is expressed preferentially at early stages of cell transformation. We are now testing this possibility by quantifying the levels of murine Trop2 in a larger set of murine cell lines at different stages of tumor progression and by comparing the growth and invasion properties of tumor cells that normally do not express Trop2 when they are transfected with the murine gene.

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