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Identification of tetratricopeptide repeat domain 9, a hormonally regulated protein ☆

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Abstract

Tetratricopeptide repeat domain 9 (TTC9) mRNA was drastically up-regulated by progesterone in progesterone receptor-transfected breast cancer cells MDA-MB-231. This up-regulation is coupled with progesterone-mediated growth inhibition and induction of focal adhesion. We have generated mouse polyclonal antibody against a predicted 222 aa TTC9 protein and identified a 25 kDa TTC9 protein that is widely expressed in human tissues, with the highest expression in the brain. Immunostaining and cell fractionation studies revealed that TTC9 is predominantly localized to the endoplasmic reticulum. The level of TTC9 protein in MCF-7 cells is regulated by various factors and chemical reagents including estrogen, progesterone, growth factors, ICI182,780, and p38 kinase inhibitor SB203580. Growth factor-induced TTC9 protein expression was inhibited by estrogen and abolished by ERK inhibitor PD98059. Though the function of TTC9 is not yet clear, the susceptibility of its protein level to biological and chemical agents suggests that TTC9 is a biologically significant protein.

Keywords: Tetratricopeptide repeat domain 9; Breast cancer; Endoplasmic reticulum; Growth factors; Steroid hormones

Estrogen and progesterone are critical for the development of normal mammary glands. They are also implicated in the development of breast cancer [1]. It is well-established that estrogen stimulates the proliferation of estrogen receptor (ER)-positive breast cancers and anti-estrogens are the frontline therapy for hormone-dependent breast cancers [2]. Although the importance of progesterone in breast cancer biology has been increasingly recognized, the effect of the hormone depends on the cell models being studied. We have previously reported that progesterone significantly inhibited cell proliferation and induced remarkable cell spreading and focal adhesion in PR-transfected MDA-MB-231 cells which were originally ER- and PR-negative [3,4]. These effects were coupled with a drastic up-regulation of the mRNA of a hypothetical protein tetratricopeptide repeat domain 9 (TTC9) in the cells. We hypothesized that TTC9 mRNA was actively translated and the protein may play some roles in mediating the function of PR on cell growth and focal adhesion.

TTC9 was first reported as a hypothetical protein KIAA0227 by Nagase et al. [5] based on the sequence analysis of a cDNA clone isolated from a brain cDNA library. It was also reported later by the National Institutes of MGC Program [6]. It is now referred to as TTC9 because of its sequence homology to a family of TTC proteins which contains tetratricopeptide repeat (TPR) domains. Human TTC9 is located on chromosome 14q24.2. Its transcript sizes are predicted to be 5217 and 2567 bp, respectively (http://www.ensembl.org), both of which encode a predicted protein of 336 amino acids (aa). The updated NCBI entry (GenBank Accession No. XM_027236.5)

☆ *Abbreviations:* TTC9, tetratricopeptide repeat domain 9; ER, estrogen receptor; PR, progesterone receptor; TPR, tetratricopeptide repeat; RT, reverse transcription; Ab, antibody; Aldo, aldosterone; Dex, dexamethasone; E2, estradiol-17β; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; DCC-FCS, dextran charcoal-stripped fetal calf serum.

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predicted a 5453 bp transcript that encodes a 415 aa protein. Using Vector NTI software, we deduced the ORF of TTC9 mRNA (GenBank Accession No. XM_027236.5) to be from nucleotide 580 to 1248, encoding a protein of 222 aa that contains 3 TPR domains at its carboxyl terminus. Blast searches using the amino acid sequence 1–51, which is outside of the TPR domains of TTC9, yielded no sequence similarity to any protein domains in the database, suggesting that TTC9 is a unique novel protein.

We have generated a mouse polyclonal antibody against the 222 aa TTC9 protein expressed in *Escherichia coli*. This report describes its identification, tissue expression, cellular localization, and the regulation of TTC9 expression by steroid hormones and growth factors.

Materials and methods

Cell line and reagents. MCF-7 cells were obtained from ATCC in 1995 at the passage of 147. Cells were routinely maintained in DMEM with phenol-red and supplemented with 7.5% fetal calf serum (FCS), 2 mM glutamine, and 40 mg/l gentamicin. For all the experiments on the effects of steroid compounds and growth factors, cells were grown in phenol-red-free DMEM containing 2 mM glutamine, 40 mg/l gentamicin, and 5% dextran charcoal-treated FCS. Cells were treated with various steroid compounds from 1000-fold stock in ethanol. Treatment controls received 0.1% ethanol only. SB203580 and PD98059 were prepared as 1000-fold stock in DMSO. Treatment controls received 0.1% DMSO only. Growth factors were added to the cell culture from 1000 stock in ddH_2O.

Chemicals. Progesterone, dexamethasone, aldosterone, estradiol-17β, basic fibroblast growth factor (bFGF), SB203580, PD98059, and FITC-conjugated anti-mouse IgG were obtained from Sigma Chemical Co. (St. Louis, MO). Epidermal growth factor (EGF) was obtained from ProSpecTany TechnoGene Ltd., Israel. ICI182,780 was bought from Tocris Bioscience (Ellisville, MO). Antibody for GAPDH was obtained from Amersham Biosciences (Buckinghamshire, UK). Antibody for ERK was from BD Transduction Laboratories (San Jose, CA). The hexyl ester of rhodamine B was obtained from Molecular Probes, Inc. (Eugene, OR). All tissue culture plastics and reagents were obtained from Invitrogen (Gaithersburg, MD).

Real-time RT-PCR for TTC9 expression. Total RNA was extracted using TRIzol reagent (Invitrogen) Five micrograms of total RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). Real-time PCR was performed with SYBR Green master mix on an ABI Prism 7000 sequence detection system (PE Applied Biosystems, Foster City, CA). The primers for TTC9 are 5′-CACAT GTCTATAACGATTTCC-3′ (forward) and 5′-TGCAGGAAACAG GGGACTCTC-3′ (reverse). PCR of 36B4 gene (human acidic ribosomal phosphoprotein P0) was included in each experiment to normalize the quantity of cDNA used. The primers for 36B4 are: forward: 5′-GATTGG CTACCCAACTGTTGCA-3′; reverse: 5′-CAGGGGCAGCAGCCACA AAGGC-3′. The PCR for each gene fragment was performed in triplicate, and each primer set was repeated three times.

Northern blotting analysis. Twelve micrograms of total RNA from each sample was separated on a 1.5% agarose gel and transferred to a nylon Hybond-N membrane. [^{32}P]-labeled TTC9 probes were generated using the coding sequence (669 bp) for TTC9. The probe was hybridized to the membrane using Ultrahyb solution of Ambion, Inc (Austin, TX) and the results were analyzed using Bio-Rad Molecular Image Analyzer (Hercules, CA).

Western blotting analysis. Cell lysate of 20 μg total proteins from each sample was analyzed by Western blotting with antibodies against specific proteins. The membrane was also probed with antibody to GAPDH to monitor the amount of proteins loaded.

Protein purification of TTC9-(His)$_6$. The 669 bp TTC9 cDNA was cloned into pET-24b(+) (EMD Biosciences, Inc., Germany) for the expression of TTC9-(His)$_6$ protein in *E. coli*. TTC9-(His)$_6$ was purified using Ni–NTA–agarose column (Qiagen GmbH) and cation exchange chromatography column (Heparin FF, Amersham Biosciences) sequentially. The final purity of TTC9-(His)$_6$ was over 90%.

Generation of polyclonal antibody against TTC9. TTC9-(His)$_6$ (50 μg) was injected into BALB/C male mice in complete Freund's adjuvant for the first injection and in incomplete Freund's adjuvant for the subsequent injections. Blood was drawn from the animals two weeks after each booster and tested for antibody titer by enzyme-linked immunosorbent assays on 96-well polystyrene plates.

Immunofluorescence microscopy. Cells were first incubated with antibody to TTC9 or to calnexin, followed by incubation with FITC-conjugated anti-mouse IgG or Alexa-Fluor 647 donkey anti-goat IgG. After washing in PBS, the cells were stained in the hexyl ester of rhodamine B at 1 μg/ml (Molecular Probes, Inc., Eugene) for endoplasmic reticulum. Stained cells were viewed and photographed using a confocal laser scanning microscope (LSM 510, Carl Zeiss, Gottingen, Germany).

Cell fractionation. MCF-7 cells were lysed in buffer containing 1 M sorbitol, 10 mM Hepes (pH 7.4), and 1 mM EDTA on ice for 30 min. The lysates were then passed through 21 G needle 40× and centrifuged at 1000*g* for 20 min. The supernatant was collected and centrifuged sequentially at 12,000*g* and 100,000*g* for 1 h to obtain the fractions 12P (pellet after centrifugation at 12,000*g*, containing mitochondria), 100P (pellet after centrifugation at 100,000*g*, containing microsomes and endoplasmic reticulum), and 100S (supernatant after centrifugation at 100,000*g*, cytoplasm). Twenty micrograms total protein from each fraction was analyzed by Western blotting.

Results and discussion

Progesterone drastically up-regulated the expression of TTC9 in ABC28 cells

ABC28 cells is a subline of ER- and PR-negative MDA-MB-231 cells transfected with PR as was described in detail previously [7]. ABC28 cells express approximately ~660 fmol PR per milligram protein as determined by enzyme immunoassay (Abbott Laboratories, Abbott Park, Illinois). Western blotting analysis using antibody Ab-8 that recognizes both PR-A and PR-B (Neomarker, Fremont, CA) showed that ABC28 cells express similar levels of PR isoform A and B. Vector-transfected cell CTC15 was used as a control cell line.

TTC9 was first identified as a progesterone-regulated gene in ABC28 cells in microarray analysis. Real-time RT-PCR analysis (Fig. 1) showed that progesterone up-regulated the mRNA level of TTC9 in ABC28 cells by 22-fold after 3 h treatment. At 24 h after treatment, the relative TTC9 mRNA level in progesterone-treated cells was 56-fold higher than that in controls.

Identification of TTC9 protein and the regulation of TTC9 protein level by steroid hormones

In order to verify that the TTC9 transcript is translated into TTC9 protein, we generated a mouse polyclonal antibody against the predicted 222 aa TTC9 protein expressed in *E. Coli* with a C-terminal His-tag. The estimated

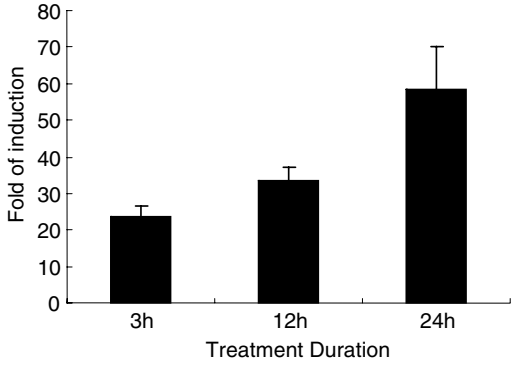

Fig. 1. Progesterone up-regulates the expression of TTC9 in ABC28 cells in a time-dependent manner. Cells were treated with control vehicle and 0.1 μM progesterone for 3, 12, and 24 h, respectively. The transcription level of TTC9 was analyzed by real-time RT-PCR. The expression level is expressed relative to vehicle-treated controls which are given the value of 1. The results are means of three replicates.

molecular weight of the 222 aa protein is 24.4 kDa. This antibody detected a protein band of ~25 kDa in ABC28 cells that is up-regulated by progesterone (Fig. 2A). Progesterone at all the doses tested increased TTC9 protein level (Fig. 2B). The effect was mediated by PR since progesterone had no effect on TTC9 protein level in vector-transfected control cells CTC15 (Fig. 2C), and progesterone antagonist RU486 completely abolished the effect of progesterone (Fig. 2D).

We have reported that MDA-MB-231 cell and its derivatives ABC28 and CTC15 express mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) [8], but their ligands aldosterone and dexamethasone had no obvious effect on TTC9 protein level in CTC15 cells (Fig. 2C). On the other hand, aldosterone and dexamethasone significantly up-regulated TTC9 protein level in ABC28 cells (Fig. 2D). This suggests that TTC9 is a PR target gene but not the target gene for MR and GR. Aldosterone and dexamethasone can cross-talk with PR to regulate the expression of PR target genes.

The hormonal regulation of TTC9 expression was then studied in ER- and PR-positive breast cancer cells MCF-7. Contrary to its effect in ABC28 cells, progesterone decreased the level of TTC9 in MCF-7 cells (Fig. 3A). This is not surprising in consideration of the fact that progesterone inhibited cell proliferation in ABC28 cells [7] but mildly stimulated the proliferation of MCF-7 cells [9]. In association with their growth-stimulatory effect, estradiol-17β (E2) alone or in combination (E2 + P) also decreased the proteins level of TTC9 by approximately 50–70% following 48 h treatments. Time course experiment showed that the regulation occurred throughout the 96 h period tested with 1 nM E2 or 1 nM E2 plus 100 nM P (Fig. 3B). Furthermore, various doses of E2 (1, 10, and 100 nM) are all effective in down-regulating the TTC9 level (Fig. 3C). These hormonal regulations seem to suggest that the TTC9 level is up-regulated when the hormonal effect is growth inhibitory and vice versa.

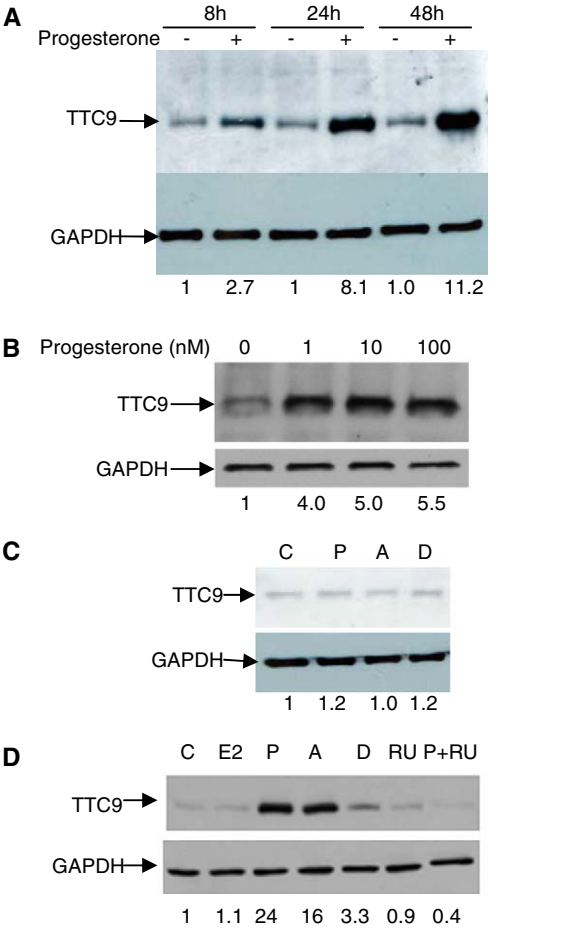

Fig. 2. Hormone regulation of TTC9 protein expression in PR-transfected MDA-MB-231 cells ABC28 and vector-transfected control cells. TTC9 protein levels were analyzed by Western blotting. The numbers associated with the blot are densitometry values of TTC9 relative to that of the vehicle-treated cells, which is given the value of 1. (A) Progesterone up-regulated TTC9 expression in a time-dependent manner. Cell lysates were collected from ABC28 cells treated with 0.1 μM progesterone (+) or control vehicle (−) for various time periods. (B) The TTC9 protein level is up-regulated by different concentrations of progesterone in ABC28 cells. Cells were treated with 1, 10, or 100 nM progesterone for 24 h. (C) The protein level of TTC9 was not regulated by steroid hormones in CTC15 cells. Cells were treated with different hormones for 48 h. C, control vehicle; P, 0.1 μM progesterone; A, 0.1 μM aldosterone; and D, 0.1 μM dexamethasone. (D) Effects of different hormones on TTC9 protein expression in ABC28 cells following 48 h treatment. RU, 0.1 μM RU486; P + RU, 1 nM progesterone plus 0.1 μM RU486.

We have attempted to knock down TTC9 level with siRNA to see if there is a reversal of progesterone-mediated effect on growth inhibition and induction of focal adhesion. Though we were able to achieve 60–70% TTC9 knock down at the protein level, there were no detectable changes on progesterone-mediated growth inhibition and focal adhesion. It is possible that the remaining TTC9 is sufficient to fulfill its cellular roles in these two aspects, or TTC9 is involved in other aspects of cellular function that remain to be discovered.

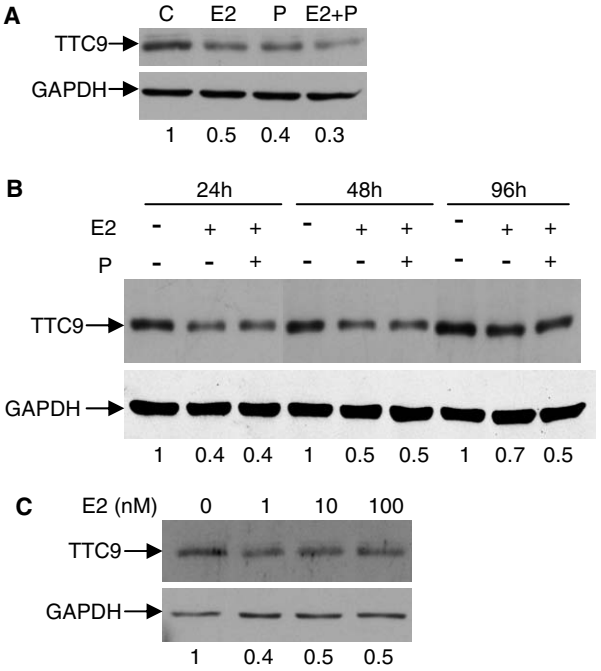

Fig. 3. TTC9 protein expression was hormonally regulated in MCF-7 cells as analyzed by Western blotting. The numbers associated with each blot are densitometry values of TTC9 relative to that of the vehicle-treated cells, which is given the value of 1. (A) MCF-7 were treated with control vehicle (C), 1 nM estradiol-17β (E2), 0.1 μM progesterone (P) or 1 nM estradiol-17β together with 0.1 μM progesterone (E2 + P) for 48 h. (B) MCF-7 cells were treated with C, E2, and E2 + P for 24, 48, and 96 h. The treatment medium was changed after 48 h in culture. (C) Various concentrations of E2 decreased the TTC9 level in MCF-7 cells. MCF-7 cells were treated with 1, 10, or 100 nM E2 for 48 h.

TTC9 is serum and growth factor inducible

Serum and growth factors are known to stimulate cell growth. The effects of these factors on TTC9 level were tested in order to see if there is any negative association between cell growth and TTC9 expression. DCC-FCS at 5%, 10%, and 20% enhanced the levels of TTC9 protein in MCF-7 cells in a concentration-dependent manner (Fig. 4A). Interestingly, FCS without DCC treatment (which removed the serum steroid hormones) at the same concentrations had little effect on TTC9 protein expression. We postulated that this is due to the antagonistic effect of serum estrogen which has been shown to decrease TTC9 level in MCF-7 cells. Indeed, E2 was able to abolish the effect of DCC-FCS on the up-regulation of TTC9 protein (Fig. 4B).

Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) also enhanced the protein level of TTC9 by 3- to 5-fold after 48 h treatment (Fig. 4C). Intriguingly, E2 was also able to inhibit the effect of EGF and bFGF (Fig. 4D). This effect of E2 is perhaps through a similar mechanism as its effect on DCC-FCS-induced increase of TTC9. It is possible that the effect of DCC-FCS on the increase of TTC9 level may be mediated through serum growth factors.

Anti-estrogen ICI182,780 inhibits cell proliferation of MCF-7 cells [10]. It up-regulated TTC9 protein level and abolished the effect of E2 in decreasing the TTC9 level (Fig. 4D). Furthermore, ICI182,780 exerted additive effect on EGF- and bFGF-induced TTC9 protein expression when it was added together with the growth factors.

It is to be noted that the Western blots in Fig. 4C, D, and 5 revealed an additional light band of higher molecular weight than the major band of approximately 25 kDa, whereas earlier figures showed one distinct band of TTC9. The difference may be due to different batches of antibodies from different mice. The identity of the additional band of higher molecular weight is not known but it appears to be also regulated by estrogen and growth factors. We do not have evidence to suggest that the upper band is post-translational modified form of TTC9.

Growth factor-induced TTC9 expression is via the activation of ERK1/2 signaling pathway

Inhibition of ERK phosphorylation by PD98059 did not have notable effect on TTC9 protein level (Fig. 5A). However, the effect of EGF and bFGF was abolished by the concurrent treatment with PD98059. This suggests that growth factor-induced TTC9 expression is via the activation of ERK1/2 signaling pathway. On the other hand, p38 kinase inhibitor SB203580 enhanced TTC9 level alone and the effects of SB203580 and growth factors are synergistic in increasing the TTC9 level (Fig. 5B).

The ERK cascade is one of the central pathways in growth factor-mediated signaling [11]. It is not surprising that growth factor-mediated increase of TTC9 protein level is abolished by the ERK inhibitor PD98059. However, the notion that TTC9 is downstream of ERK signaling seems to contradict to the report that estrogen is known to transiently activate ERK in MCF-7 cells [12] yet it down-regulated the level of TTC9 and it inhibited the effect of growth factors. Furthermore, anti-estrogen ICI182,780 was able to abolish E2-mediated activation of ERK [12], yet it enhanced the level of TTC9 when it was added alone or together with E2. It is likely that other cellular effects of E2 cancelled ERK-mediated signaling and the regulations by growth factors and estrogen on TTC9 protein level are mediated through separate pathways.

Subcellular localization of TTC9

Cell fractionation study was performed using MCF-7 cells (Fig. 6A). Mitochondria are present mainly in 12,000*g* pellet as evidenced by the presence of mitochondria marker cytochrome *c* oxidase subunit IV (COX IV). On the other hand, endoplasmic reticulum is present in both the 12,000*g* pellet and 100,000*g* pellet as shown by the presence of calnexin, a well-known endoplasmic reticulum marker [13]. TTC9 is present in both the 12,000*g* pellet and the 100,000*g* pellet fractions. Since both fractions contain endoplasmic reticulum, we can conclude that TTC9 is

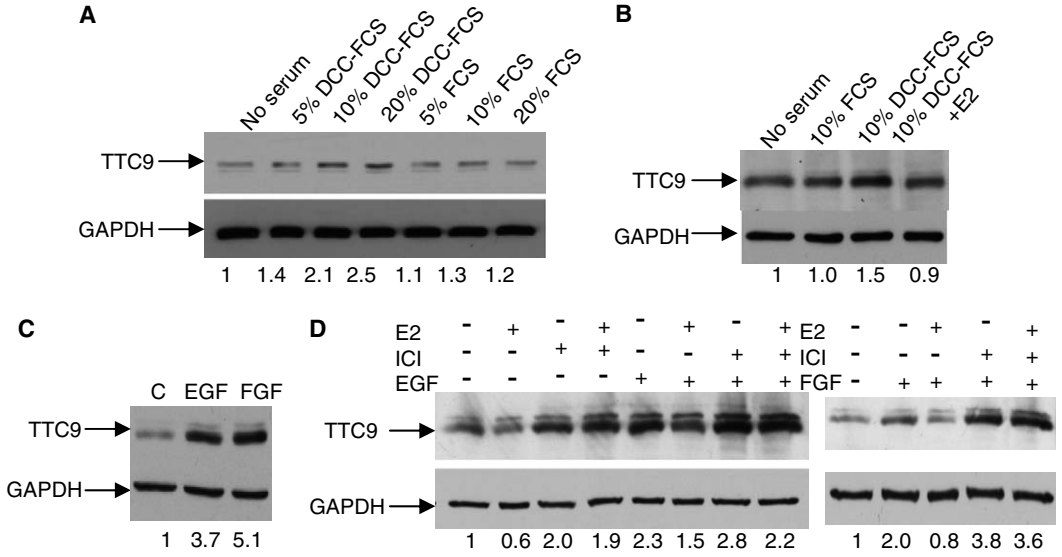

Fig. 4. TTC9 protein was serum and growth factor inducible in MCF-7 cells. Total cell lysates was collected following various treatments and subjected to Western blotting analysis. The numbers associated with each blot are densitometry values of TTC9 relative to that of the vehicle-treated cells, which is given the value of 1. (A) The effect of serum on TTC9 protein level. MCF-7 cells were grown in serum-free medium and in media containing various concentrations of DCC-FCS or FCS as indicated for 48 h. (B) The up-regulation of TTC9 protein level by 10% DCC-FCS was abolished by 1 nM E2. MCF-7 cells were treated for 48 h before the cell lysate were collected for Western Blotting analysis. (C) EGF and bFGF increased the level of TTC9 protein in MCF-7 cells. MCF-7 cells were treated with 100 ng/ml EGF and 50 ng/ml bFGF, respectively, for 48 h. (D) The up-regulation of TTC9 protein level by growth factors was counteracted by E2 but not by ICI182,780. MCF-7 cells were treated with 100 ng/ml EGF, 50 ng/ml FGF, together with either 1 nM E2, 0.1 μM ICI182,780 (ICI), or both as indicated for 48 h.

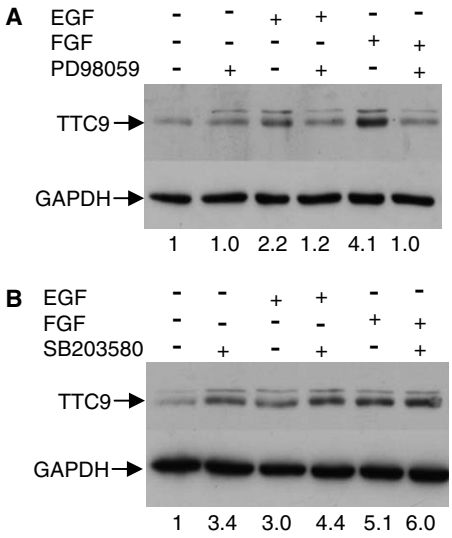

Fig. 5. Growth factor-induced TTC9 expression is abolished by ERK inhibitor PD98059 (A) but not by p38 kinase inhibitor SB203580 (B) in MCF-7 cells. MCF-7 cells were treated with 100 ng/ml EGF, 50 ng/ml FGF in the presence or absence of either PD98059 (50 μM) or SB203580 (10 μM) as indicated for 48 h. Both inhibitors were added to the cell medium 30 min before the addition of growth factors. TTC9 expression levels were analyzed by Western blotting. The numbers associated with each blot are densitometry values of TTC9 relative to that of the vehicle-treated cells, which is given the value of 1.

concentrated mainly to the endoplasmic reticulum. Nonetheless, a lighter band of TTC9 was also observed in the 100,000*g* supernatant fraction, which is the cytoplasmic fraction.

The subcellular localization of TTC9 in MCF-7 cells was also examined by immunostaining. Hexyl ester of Rhodamine B is known to stain endoplasmic reticulum at high concentrations [14]. Since Rhodamine B (Fig. 6B, green) perfectly overlaps with endoplasmic reticulum marker protein calnexin (red), we used Rhodamine B as an endoplasmic reticulum marker to avoid the non-specific staining associated with the use of antibody to calnexin. The immunostaining of TTC9 (green) largely overlaps with the staining of Rhodamine B (red, Fig. 6B). This further supports the results of cell fractionation studies which localize TTC9 mainly to the endoplasmic reticulum.

Nonetheless, TTC9 does not contain any endoplasmic reticulum localization signal. Hydropathy plot [15] revealed that human TTC9 is a soluble protein with no transmembrane domain (Fig. 7). It is plausible that TTC9 is anchored to the endoplasmic reticulum membrane through protein modification. There are several potential sites (aa. 5–10, 33–38, 36–41, and 42–47) for N-myristoylation in this protein. Attachment of the myristoyl residue provides hydrophobicity to facilitate the partitioning of proteins to cellular membranes [16,17]. TTC9 may also be targeted to the endoplasmic reticulum by interaction with other proteins. It has been shown that TPR containing protein FKBP38 can be translocated to endoplasmic reticulum from mitochondria by forming macromolecular complex with presenilins 1 and 2 [18]. ScanProsite analysis of TTC9 indicated the presence of two TPR domains in between aa. 128 and 197. In addition, a tetratricopeptide-like repeat, TPR_4 domain, was indicated between aa. 50 and 85. Interaction of these TPR domains with other endo-

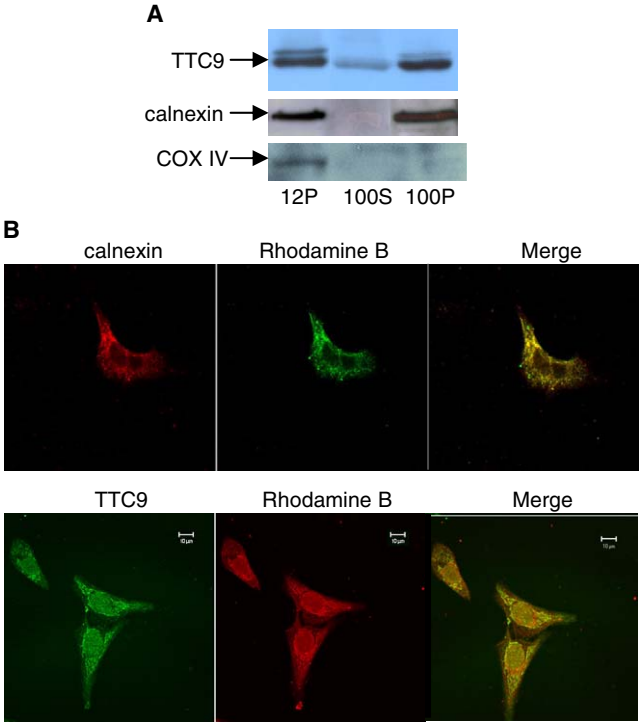

Fig. 6. Subcellular localization of TTC9. (A) Cells were subfractionated as described in Materials and methods. 12P is the pellet fraction after centrifugation at 12,000*g*; 100P is the pellet fraction after centrifugation at 100,000*g*; 100S is the supernatant after centrifugation at 100,000*g*. Calnexin and COX IV were used as markers for endoplasmic reticulum and mitochondria, respectively. (B) MCF-7 cells were grown on glass coverslips and stained for calnexin together with the hexyl ester of Rhodamine B (top panel), or stained for TTC9 together with the hexyl ester of Rhodamine B (lower panel). Calnexin was probed with anti-calnexin antibody and detected by Alexa-Fluor 647 donkey anti-goat IgG (red). TTC9 was probed with TTC9 antibody and detected by FITC-conjugated anti-mouse IgG (green). Rhodamine B staining completely overlaps with that of calnexin, supporting its use as an endoplasmic reticulum stain at the concentration of 1 μg/ml. The images were obtained using Zeiss confocal laser scanning microscope model LSM510 (Bar, 10 μm).

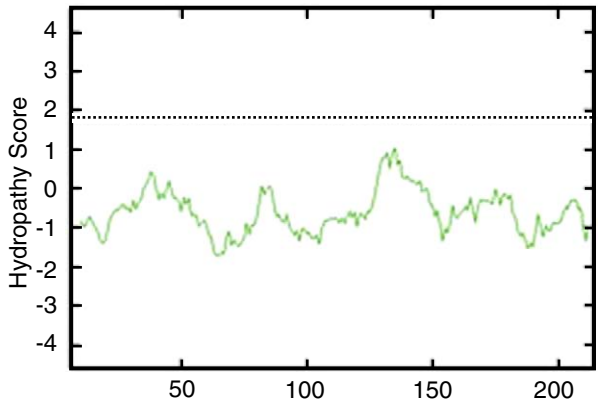

Fig. 7. Kyte–Doolittle Hydropathy Plot of human TTC9 protein indicated that TTC9 is a soluble protein. Hydropathy blot of human TTC9 protein was generated with window size at 19. *X* axis represents the amino acids position in the protein sequence. The region with hydrophobocity value above the dotted line is considered as putative transmembrane domains.

plasmic reticulum-localized proteins may facilitate the localization of TTC9 to the endoplasmic reticulum.

Identification of TTC9 transcripts and protein in human tissue

Northern blotting analysis using specific [^{32}P]-labeled TTC9 probe (Fig. 8A) identified a band of ~2.5 kb that is up-regulated by progesterone in ABC28 cells. This probably corresponds to the shorter transcript of 2567 bp predicted by Ensembl. The 2.5 kb transcripts were also the only band detected in MCF-7, T47D, and ZR-75.1 cells (data not shown).

An estimated 5.1 kb transcript, as well as a 2.5 kb transcript, was detected in some of the 12 tissues of the commercial mRNA blot (Fig. 8B). An additional 5.9 kb band was also detected in cardiac and skeletal muscle tissues. TTC9 gene expression is the highest in brain tissue. However, the RNA in some of the tissues such as the colon looks degraded. It is difficult to assess the relative expression of TTC9 among various tissues using this blot.

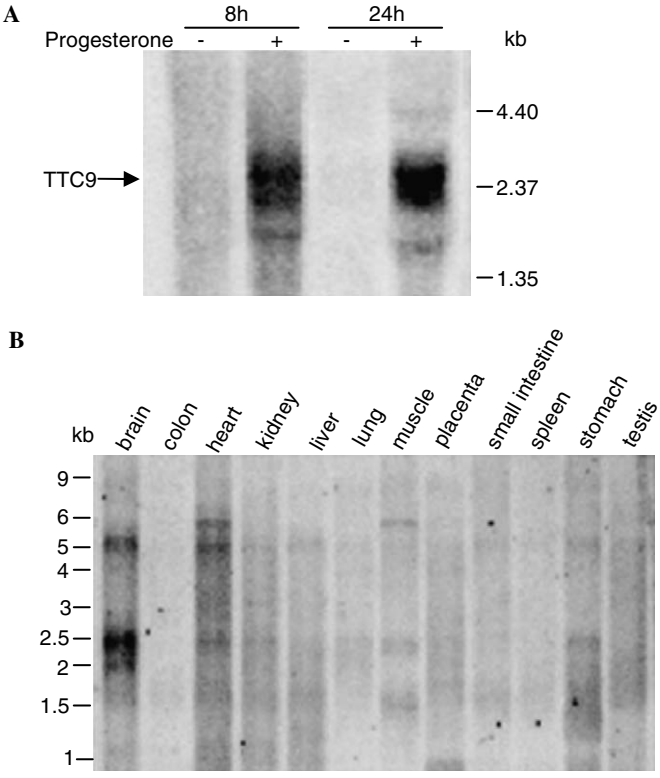

Fig. 8. Identification of TTC9 transcripts in the commercial human tissue mRNA blot. (A) ABC28 cells were treated with 0.1 μM progesterone (+) or control vehicle (−) for various time periods. Total RNA was analyzed by Northern blotting as described in Materials and methods. Molecular size ladders of RNA were indicated on the right. (B) TTC9 mRNA levels were determined using [^{32}P]-labeled TTC9 probe as described in Materials and methods. The probe detected transcripts of three different sizes in different human tissues, i.e., 2.5, 5.1, and 5.9 kb. Human Northern RNA blot was obtained from OriGene Technologies, Inc. (Rockville, MD).

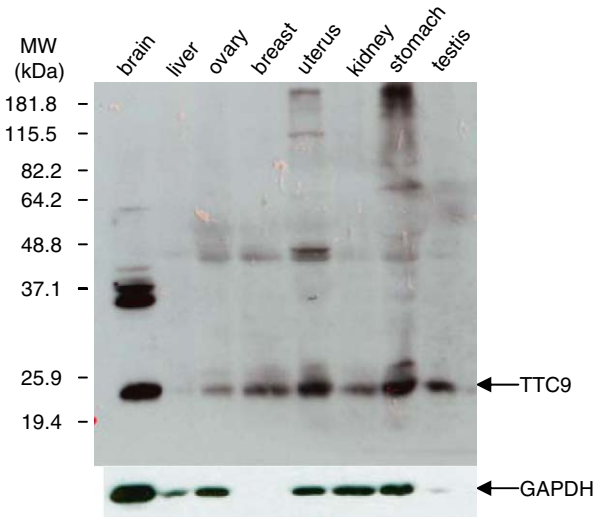

Fig. 9. Identification of TTC9 protein in the commercial human tissue blot. TTC9 protein expression pattern in eight human normal tissues. Human tissue blot was bought from ProSci Inc. (Poway, CA). The expression of TTC9 protein was analyzed by mouse anti-TTC9 polyclonal antibody. Molecular weight markers (left) indicated the TTC9 band appeared at ~25 kDa.

The antibody identified a ~25 kDa protein in human tissues of a commercial Western blot (Fig. 9). It also detected a prominent double band of 36–37 kDa proteins in human brain but their identity remains to be investigated. In accordance with the mRNA expression, TTC9 protein is also most abundant in the brain tissue. Though liver tissue expresses little TTC9, the protein seems to be ubiquitously expressed in all the tissues studied.

In summary, this study identified a hitherto unconfirmed hypothetical protein TTC9 that is ubiquitously expressed in human tissues, with the highest expression in the brain. Though human tissues appear to express three mRNA variants, breast cancer cells expressed only one mRNA transcript (2.5 kb). The TTC9 protein is predominantly concentrated to the endoplasmic reticulum, and its expression was regulated by a number of factors including steroid hormones, serum, and growth factors. ERK pathway appears to be involved in growth factor-mediated regulation of TTC9 expression. Sequence alignment of human TTC9 with that in mouse, rat, and chimpanzee revealed over 90% homology, suggesting that the protein is evolutionarily well-conserved among the species. We are currently screening for TTC9 interacting proteins and hope that this work will shed more light on its possible biological function.

Acknowledgments

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