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Research Article

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Expression of TCTP-Related Genes

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Abstract

Translationally controlled tumor protein (TCTP) encoded by *TPT1* gene is a multifunctional protein involved in fundamental cellular processes of cell-cycle progression, proliferation, survival, malignant transformation, and regulation of pluripotency and cancer stem cells, as well as allergic inflammation. Here we report the cloning of four TCTP-related cDNAs from human peripheral blood mononuclear cells. These genes lack introns, suggesting that they are pseudogenes generated by retrotransposon-mediated integration of *TPT1* gene. However, some of them are expressed at extremely low levels in limited tissues and hematopoietic cells. None of the four related proteins is expected to bind IgE, which is required for TCTP's proallergic reactions. These results collectively suggest that they genes play negligible biologic roles in humans.

Keywords

TPT1 gene; Translationally controlled tumor protein; Pseudogenes

Introduction

The *TPT1* gene encoding translationally controlled tumor protein (TCTP) was discovered as a gene highly expressed in tumor cells [1-3]. Despite its name, TCTP expression is subject to both transcriptional and translational control [4-7]. TCTP within a cell plays a critical role in the fundamental processes of cell-cycle progression, proliferation, survival, malignant transformation and regulation of pluripotency [8-10]. TCTP, a Ca^{2+}-binding protein [11-14], is involved in the elongation step of protein synthesis by interacting with both eEF1A (a small GTPase) and eEF1B β (a guanine nucleotide exchange factor) [15-17]. *Drosophila* TCTP acts as the guanine nucleotide exchange factor for Rheb (Ras homologue enriched in brain), a GTPase of the Ras superfamily that regulates the TSC1-mTOR pathway [18]. Downregulation of TCTP expression induces a reversal of tumor cells into a normal cell phenotype [19,20]. TCTP is an anti-apoptotic protein: TCTP interacts with Mcl-1 [21,22] and Bcl-xL [23], anti-apoptotic members of the Bcl-2 family. Recent studies identified mutual antagonism between p53 tumor suppressor and TCTP, with TCTP promoting p53 degradation via MDM2-mediated ubiquitination of p53 and with p53 directly repressing TCTP transcription [24,25]. Another conserved property of TCTP is its interaction with microtubules [26] and mitochondria [27]. TCTP is a microtubule-binding and -stabilizing protein, and its phosphorylation by polo-like kinase decreases the microtubule-stabilizing activity of TCTP and promotes the increase in microtubule dynamics that occurs after metaphase [28]. Probably because of the above-mentioned roles of TCTP in the fundamental cellular processes, TCTP knockout mice are embryonically lethal [29,30].

Despite the lack of a signal sequence, TCTP can be secreted and the secreted protein is also known as histamine-releasing factor (HRF). HRF can stimulate histamine release and IL-4 and IL-13 production from IgE-sensitized basophils and mast cells [31,32]. HRF-like activities were found in bodily fluids during allergic reactions, implicating HRF in allergic diseases [33,34]. We recently found that ~25% of IgE molecules can bind to HRF via their Fab interactions with two binding sites within the HRF molecule, N19 and H3 [35]. The use of peptide inhibitors that block HRF-IgE interactions revealed an essential role for HRF in promoting skin hypersensitivity and airway inflammation [35].

The chromosomal localization of human [36,37] and mouse [38] *TPT1* genes has been described. *TPT1*-derived pseudogenes were found in mouse [38], rabbit and human [39] genomes. In this study, we isolated four *TPT1*-related transcripts and studied their transcription in various human cell types and tissues.

Materials and Methods

Cloning of TCTP-related cDNAs

Candidate human TCTP-related nucleotide sequences were searched in Human genomic plus transcript database with the BLAST algorithm. mRNA expression of the TCTP-related genes was analyzed by RT-PCR and only genes whose mRNA expression was detected were cloned. Human TCTP-related cDNAs were amplified by RT-PCR using the primers listed in Table 1 and ligated in pCR-Blunt II-TOPO vector (Invitrogen). The cDNA sequences were analyzed using M13 reverse and M13 forward (-20) primers.

Sequence analysis

Alignments of nucleotide and protein sequences of human TCTP-related molecules were done by Genetyx software (Version 11). Molecular phylogenetic trees of the TCTP-related nucleotides and proteins were constructed by UPGMA algorithm [40-43].

mRNA expression

An equal amount of total RNA (1 μg) was used for reverse transcription reaction. Quantitative RT-PCR was performed as follows: cDNA (10 ng) was amplified in a 25 μl reaction volume containing SYBR Premix Ex Taq II (TAKARA) and 0.4 μM forward and reverse primers listed in Table 2 using Applied Biosystems 7500 real-time PCR system. PCR conditions: 95°C for 30 sec, followed by 40 amplification cycles (TCTP & TCTP2: 95°C for 15 sec; 60°C 1 min, TCTP3 & 4: 95°C for 15 sec; 57°C for 30 sec; 72°C for 1 min, TCTP5: 95°C for 15 sec; 53°C for 30 sec, 60°C for 1 min). All amplified PCR products yielded a single melting peak. Absolute expression levels of the TCTP-related molecules were determined using each cDNA as a standard.

Statistical analysis

Statistical analysis was performed using Student's t test. *P* values of <0.05 were considered statistically significant.

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Table 1: List of primers used to amplify human TCTP-related cDNAs.

	5' primer	3' prime
TCTP2	AAAAGGATCCATGGTCAGTAGGACAGAAGG	AAAAGAATTCTTAACATTTTTCCGTTTCTA
TCTP3	AAAAGGATCCATGATTATCTACCGCGACCT	AAAAGAATTCTGCCAAATTTGTTAACATTT
TCTP4	AAAAGGATCCATGGAAACCGTCATCATGAT	AAAAGAATTCTCATGTTTTCTGGTAGTTTT
TCTP5	AAAAGGATCCATGATTATCTATCCAGACCT	AAAAGAATTCTTAATAATTTTTCATTTCCAA

Table 2: List of forward and reverse primers used.

TCTP forward	ATCGCGGACGGGTTGTGCCT
TCTP2 forward	ATTGTGGACGGGCTGTGCTG
TCTP3 forward	ATCATGGACGGGCTGTACCT
TCTP4 forward	ATTGCAGACGGGCTGCGCCT
TCTP5 forward	AGTGAGATTTACAAGAA
Common reverse	AGTGATTACTGTGCTTTC

Results

The human *TPT1* gene (NM_003295.2) on chromosome 13q14.13 [37] is the canonical *TCTP* gene. We have isolated 4 related genes located on different chromosomes by PCR from a cDNA preparation derived from human PBMCs (Figure 1): *TCTP2* (XM_001718916.1) on chromosome 9q33.1; *TCTP3* (XR_038938.1) on chromosome 6q24.2; *TCTP4* (NM_207422.1) chromosome Xq13.1; *TCTP5* (AL359851.19) chromosome Xq24-25. These genes are highly homologous to TCTP: TCTP2, 76.2%; TCTP3, 29.1%; TCTP4, 67.2%; TCTP5, 41.9% identical at the amino acid level (Figure 2 and Table 3). TCTP2 lacks the N-terminal 34 residues, but the rest of the amino acid sequence is similar to TCTP except for the replacement of 5 residues. Thus, TCTP2 lacks the IgE-binding N19 region, but has the other HRF-binding H3 domain that is identical to TCTP. TCTP3 is only 55 residues long, corresponding to the TCTP's N-terminus with 5-residue replacements. Thus, TCTP3 retains the N19 sequence, but lacks the H3 domain. TCTP4 can encode a 140-residue protein with a 5-residue N-terminal extension and the loss of homology after the stretch of the sequence of NYQ. TCTP4 also has 14-residue replacements, including 5 residues in the N19 region, compared with TCTP. TCTP5 is the least homologous member with two internal deletions of four residues corresponding to residues14-17 of TCTP, which is within the N19 region, and one residue corresponding to residue 72 of TCTP and an insertion of 5 nucleotides after the MKS stretch corresponding to residues 96-98 of TCTP, resulting in a frame shift. In addition to these changes, TCTP5 has 22-residue replacements in the 1-98 segment of TCTP.

Lacking introns unlike *TPT1*, they must have been generated by retrotransposon-mediated integration of the *TPT1* gene and subsequent mutation during evolution. Processed pseudogenes of *TCTP* were also found in the rabbit and mouse genomes [6,38]. Pseudogenes are generally considered inactive, but some studies report the transcription of processed pseudogenes [40-42]. This seems to be the case for some rabbit pseudogenes of *TPT1* [39]. Therefore, we determined whether these related sequences are expressed in human tissues and cells. All tested human tissues, including brain, heart, lung, liver, spleen, kidney, thymus, blood, tonsil, trachea, pancreas, small intestine, placenta, fetal liver, fetal spleen and fetal thymus, well expressed *TCTP* mRNA (encoded by *TPT1*) (Figure 3). However, no tissues expressed *TCTP2*. *TCTP3* was expressed only in lung and thymus. *TCTP4* was expressed by brain, lung, liver, spleen, kidney, thymus, placenta, and fetal liver. *TCTP5* was expressed by brain, spleen, kidney, thymus, blood, and fetal liver. Interestingly, thymus expressed *TCTP3*, *TCTP4*, and *TCTP5* at the highest levels among the tested tissues. However, their levels were about one order of magnitude lower or less than that of *TCTP*.

Since we are interested in potential interference with or enhancement of TCTP's role by the TCTP-related proteins in allergic and hematologic diseases, we tested the mRNA expression of *TCTP* and its related pseudogenes in hematopoietic human cell lines including T cells (Jurkat and ATL-1K), B cells (NALM6 and RAMOS), mast cells (HMC-1), monocytes (MOLM13, THP-1 and U937), lymphoblasts (K562), promyelocytes (HL-60), megakaryoblasts (UT-7), and erythroblasts (TF-1 and HEL). Consistent with previous studies, *TCTP* mRNA was expressed at high levels by all cell lines (Figure 4). By contrast, *TCTP2* was not expressed by any cell lines, which is in line with the tissue expression results. Compared with *TCTP* mRNA, *TCTP3* was expressed at ≥1000-fold lower levels by Jurkat and THP-1 cells. *TCTP4* was expressed at ≥100-fold lower levels by Jurkat, NALM6, HMC-1, MOLM3, and THP-1 cells. *TCTP5* was expressed at >28-fold lower levels by Jurkat (>620-fold) and HMC-1 (28.8-fold) cells.

Discussion

This study demonstrates that several *TCTP*-related intronless sequences are present in the human genome. These *TCTP*-related pseudogenes, particularly *TCTP2*, are expressed very poorly, i.e., at least 100-fold lower than *TCTP*, except for *TCTP5* in HMC-1 mast cells, which is expressed around one thirtieth of *TCTP* level. Such a low and limited expression almost guarantees negligible biological importance of these gene products. Given the importance of HRF homodimer, whose disulfide bonding involves Cys172, in activation of mast cells, it is an important observation that the least expressed *TCTP2* is only one with an equivalent Cys residue. As inflammations induced by HRF-IgE interactions are suppressed by single HRF inhibitors such as GST-N19 and GST-H3 [35], both IgE-binding sites in N19 and H3 regions are required for HRF's biologic functions. Among TCTP-related proteins, TCTP4 alone has both N19 and H3 regions. However, TCTP4's N19 region would not bind IgE, as it has 5-residue replacements. These results collectively suggest that *TCTP*-related pseudogenes *TCTP2-5* do not play significant biologic functions within and outside the cell.

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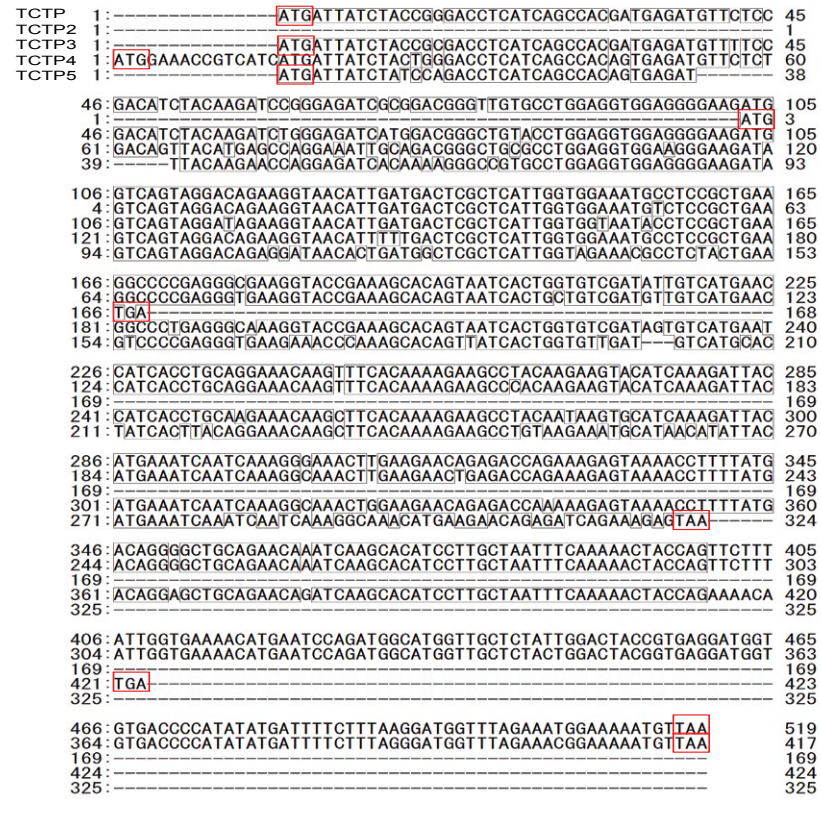
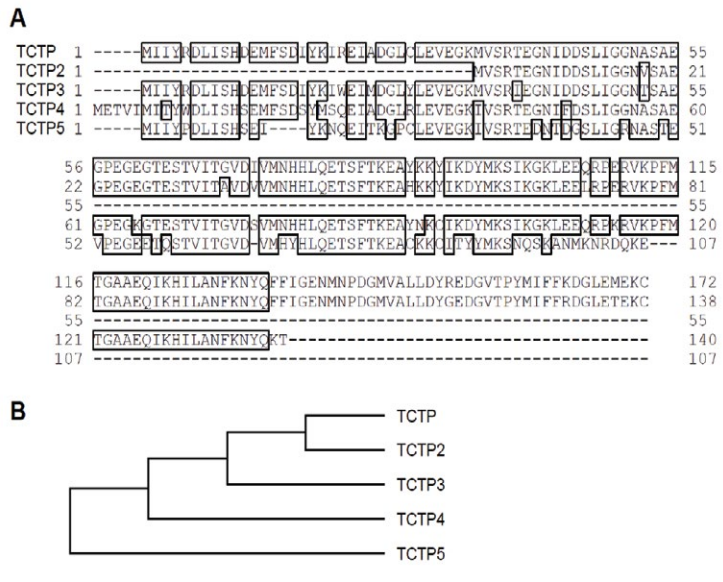


Figure 1: Nucleotide sequences of human TCTP and TCTP-related cDNAs.



A: Amino acid sequences of TCTP-related cDNA products

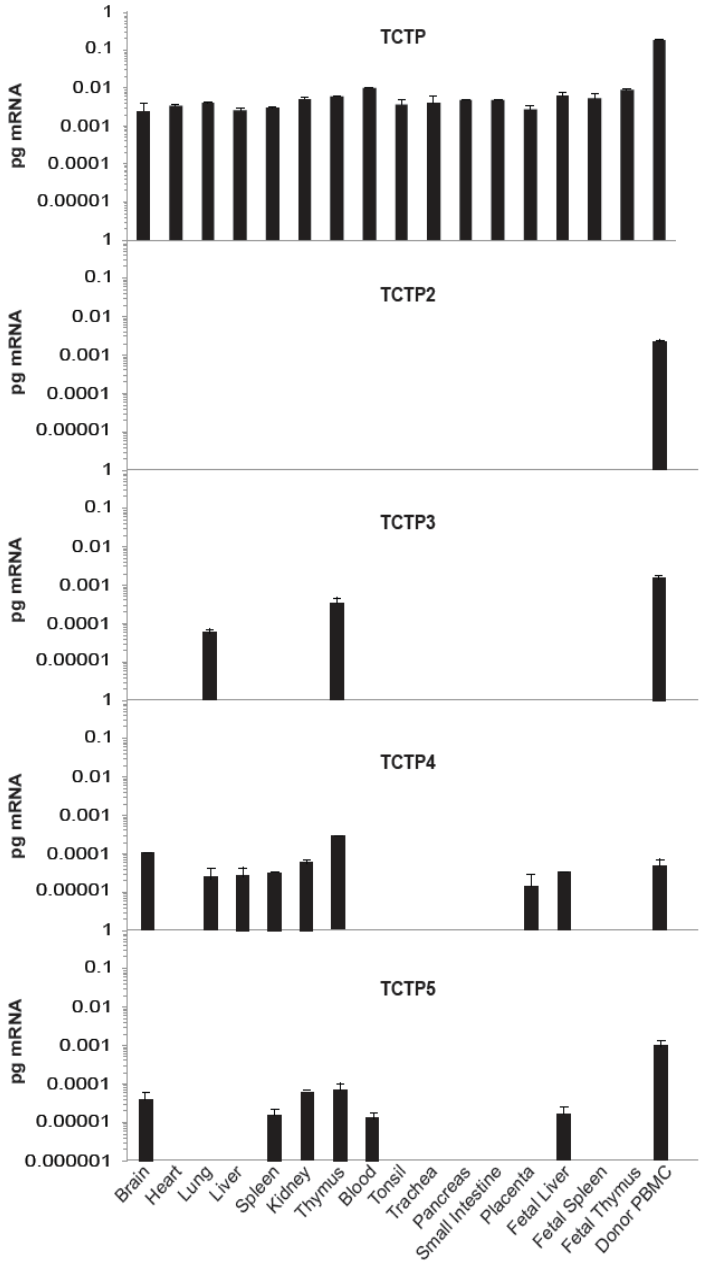
B: Molecular phylogenetic trees of the TCTP-related proteins (D) were shown

Figure 2: Sequence alignment of TCTP-related genes.

Table 3: Table showing percentage of amino acid homology.

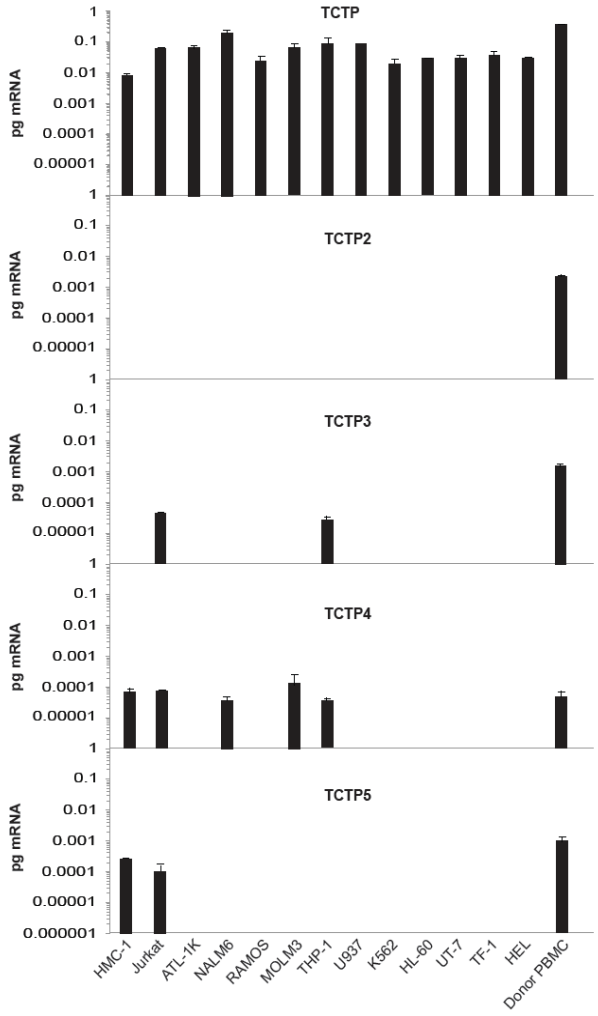
Name	Gene ID	# of AA	Chromosome	% homology*
TCTP	NM_003295	172 aa	13q14.13	N/A
TCTP2	XM_001718916.1	138 aa	9	76.2%
TCTP3	XR_038948.1	55 aa	6	29.1%
TCTP4	NM_207422.1	140 aa	Xq13.1	67.2%
TCTP5	AL359851.15	107 aa	Xq24-25	41.9%

*Amino acid identity was calculated against 172 residues in TCTP, except that TCTP4's homology was calculated against the denominator of 177 with its 5 residue N-terminal extension.



cDNAs were synthesized from human tissue RNAs, and the expression of TCTP -related molecules was analyzed by qRT-PCR. The Data are presented as mean ± SEM

Figure 3: Expression of TCTP and related genes in human tissues.



qRT-PCR data are presented as mean ± SEM. HMC-1, mast cell leukemia cell; Jurkat, T cell leukemia cell; ATL-1K, adult T -cell leukemia cell; NALM6, acute lymphoblastic leukemia cell; RAMOS, Burkitt's lymphoma cell; MOLM3, acute lymphoblastic leukemia cell; THP-1, acute monocytic leukemia cell; U937, histiocytic lymphoma cell; K562, chromic myeloid leukemia cell; HL -60, acute promyelocytic leukemia cell; UT-7, megakaryoblastic leukemia cell line; TF-1, erythroleukemia cell; HEL, erythroleukemia cell

Figure 4: Expression of TCTP and related genes in human hematopoietic cells.

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