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Critical Roles of Translationally Controlled Tumor Protein in the Homeostasis and TCR-Mediated Proliferation of Peripheral T Cells

Peih-Shan Wu,*† Chia-Yu Yang,*† Jeffrey Jong-Young Yen,*‡ Chiang-Hung Chou,† Sung Ho Chen,† Chi-Kuang Leo Wang,† Yein-Gei Lai,† Nan-Shih Liao,*† and Hsin-Fang Yang-Yen2*†

Translationally controlled tumor protein (TCTP) is expressed throughout T cell development and prominently induced following T cell activation. However, its function(s) during these processes is unclear. Here, we demonstrated that conditional deletion of TCTP before the β selection checkpoint resulted into a partial block of thymocyte development at the double-negative (DN) 3 stage. Deletion of TCTP in the double-positive (DP) stage did not cause any significant phenotype in the thymus except a slight increase of mature CD8 single-positive (SP) thymocytes. In contrast to the very modest phenotype observed in the thymus, a significant reduction of mature T cells was observed in the peripheral lymphoid organs of these two conditional null TCTP mutant mice. Detailed analysis revealed that the latter phenotype (peripheral T cell lymphopenia) was largely due to a decreased viability of mature TCTP-deficient (TCTP−/−) T cells. Transgenic expression of the anti-apoptotic protein Bcl-2 rescued the partial block of early thymocyte development, but not peripheral T cell lymphopenia of T-lineage-specific TCTP−/− mice, suggesting that the signaling networks of TCTP in these two processes are not identical. Last, we demonstrated that TCTP−/− T cells manifested a significant defect in T cell Ag receptor (TCR)-mediated cell proliferation. Further analysis revealed that such defect was due to a marked delay in the initial cell-cycle entry of TCTP−/− T cells following TCR stimulation. Together, these results indicate that TCTP plays a very modest role in thymocyte development, but is critical for peripheral T cell maintenance and TCR-mediated cell proliferation. The Journal of Immunology, 2009, 183: 2373–2381.

Translationaly controlled tumor protein (TCTP) is an evolutionally highly conserved protein (1). The extremely high degree of sequence conservation during evolution suggests that TCTP plays an essential role in the development of various organisms. Recent genetic studies conducted both in fly and mouse systems confirmed this notion, i.e., Drosophila deficient of TCTP died at the late first instar stage (2), whereas mouse without TCTP died around E9.5-E10.5 with a severely disorganized structure (3).

TCTP is highly regulated in response to a wide range of extra-cellular signals and cellular conditions (4). In tumor cells TCTP was found to be strongly expressed and is down-regulated upon tumor reversion (5). Biochemical studies indicate that TCTP is a calcium binding protein (6) that can interact with many proteins implicated in cell growth and death controls. For example, studies on interaction with tubulin (7) and Plk (8) suggest that TCTP is involved in cell cycle control. The association of TCTP with translation elongation factors eEF1A and eEF1Bβ (9, 10) implicates a role of TCTP in protein translation. In contrast, the anti-apoptotic function of TCTP may result from its interaction with Mcl-1 (11, 12) and/or with Bcl-XL (13).

Although diverse cellular functions have been reported for TCTP, how TCTP achieves all these functions are largely unknown. Structurally, TCTP resembles the Mss4/Dss4 family of guanine nucleotide-free chaperones (1). Recently, Drosophila TCTP was reported to control cell growth and proliferation by functioning as a guanine nucleotide exchange factor (GEF) for dRheb and positively regulate downstream dS6k activity (2). In addition to its cytoplasmic roles, TCTP appeared to have extra-cellular functions, because the human homologue of TCTP (termed Histamine Releasing Factor, HRF) was found to be the component present in patients’ biological fluids that could stimulate histamine release from basophils (14). Recently, a nuclear function was further identified for TCTP, as it was demonstrated that TCTP binds to the regulatory region of the mouse oct4 gene and activates transcription of oct4 in transplanted somatic nuclei (15).

TCTP is widely expressed in many tissues and cell types (3, 13). In this study, we aimed to study TCTP functions in the T cell lineage, because TCTP was shown to be expressed in thymus and was significantly up-regulated in T cells activated by T cell Ag receptor (TCR) ligation and CD28 costimulation (13). To bypass the embryonic lethality of mice with germine deletion of the TCTP gene (3), we generated mutant mice in which the TCTP gene was conditionally deleted at two different stages during
thymocyte development. Analysis of these mutant mice revealed that TCTP plays a very modest role in thymocyte development, but is critical for peripheral T cell homeostasis and TCR-mediated cell proliferation. Possible functions of TCTP in these processes were also explored.

Materials and Methods

Antibodies

Anti-TCTP Ab was generated as previously described (12). Other Abs used in this study include Bcl-2 (Santa Cruz Biotechnology), -actin (Sigma-Aldrich); cyclin E2 and cyclin D3 (obtained from Cell Signaling).

Flow cytometry

Thymocytes, splenocytes and lymph node cells were harvested and filtered through 70 μm cell strainers (BD Falcon) in staining buffer (1X PBS, 2% FBS, 0.02% Na3citrate) to generate single-cell suspensions. This was followed by treatment of cells with ACK lysis buffer (0.15 M NH4Cl, 10 mM KHCO3, 0.1 mM Na2EDTA) to lyse RBC. Before cell surface staining with specific Abs, FcRlabeled with the indicated two dyes were included in some sets of experiments to confirm the preinjection ratio of Lck-TCTPf/d mice. To minimize any possible bias from a specific labeling dye, control and mutant cells reverse-labeled with the indicated two dyes were included in some sets of experiment.

In vivo SP thymocyte homing

In vivo SP thymocyte homing was conducted according to a published protocol (18) with some modifications. Briefly, mature (Tcrβ mice) CD4 or CD8 SP thymocytes purified from control or Lck-Cre-TCTPf/d mice were injected into 30 min at 37°C with 0.1 μM CellTracker Green CMFDA (5-chloromethylfluorescein diacetate), 20 μM CellTracker Blue CMAC (7-amino-4-chloromethylcoumarin) (Molecular Probes) or 2 μM PKH-26 (Sigma-Aldrich). After labeling, cells were washed and incubated at 37°C for additional 30 min to allow complete modification of the probe. Equal numbers of labeled cells from each cell group were then mixed in PBS, and an aliquot of this mixture was analyzed by flow cytometry to confirm the preinjection ratio of Lck-TCTPf/d to control SP thymocytes. Two to ten million cells of this cell mixture were injected into the tail vein of normal mice. Sixteen hours after injection, lymph nodes and spleens from the recipient mice were analyzed by flow cytometry for the presence of control and Lck-TCTPf/d SP thymocytes. To minimize any possible bias from a specific labeling dye, control and mutant mice cells reverse-labeled with the indicated two dyes were included in some sets of experiment.

In vivo cell survival assay

In vivo cell survival assay was conducted as previously described (19). Briefly, Mature SP thymocytes (CD4 or CD8) were purified from control or Lck-TCTPf/d mice and labeled with CFSE (Molecular Probes) for 10 min at 37°C. Three to five million of labeled cells were i.v. injected into normal mice. After 3 days, lymph nodes and spleens from the recipient mice were analyzed for the presence of CFSE-labeled cells. This assay gave a similar result to that obtained by injecting T cells into a congenic strain of mice, and following the presence of injected T cells using Abs specific for congenic markers (19).

Western blotting analysis

Subpopulations of thymocytes (DN, DP, CD4SP, and CD8SP), DN cells (DN1, DN2, DN3 and DN4), splenocytes or lymph node T cells (CD4+ or CD8+) were purified by fluorescence activated cell sorter. Purified cells were lysed in a lysis buffer (10 mM Tri [pH 7.5], 150 mM NaCl, 5 mM EDTA, 1% Triton X-100) containing 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin. Cell lysates from equal number of cells were resolved by SDS-PAGE, transferred to a PVDF membrane (Millipore), and probed with specific Abs as indicated in each figure. Immune complexes were detected with HRP-conjugated goat Abs to mouse or rabbit IgG and revealed by an ECL Western blot system (Amersham Pharmacia Biotech).

Statistical analysis

Statistical analysis was performed with unpaired, two-tailed Student’s t test using GraphPad Prism 4.0 software.

Results

Generation of T cell-specific TCTP knockout mice

TCTP is expressed in the DN, DP and CD4CD8+ or CD4CD8+ single-positive (4SP or 8SP) thymocyte subsets (Fig. 1A), suggesting that it plays a role in T cell development in the thymus. To address this issue, we generated mice whose TCTP gene was specifically deleted during the DN or DP stage of the T cell development. To do this, the Lck-Cre or CD4-Cre transgene (17) was first bred to the mutant mice carrying a germline deletion allele of the TCTP gene (TCTPf/d) (3) to generate Lck-Cre-TCTPf/d and CD4-Cre-TCTPf/d mice, respectively. These two mouse lines were then crossed with the TCTPf/f mice that carried the floxed allele of the TCTP gene (3) to generate Lck-Cre-TCTPf/f and CD4-Cre-TCTPf/f mice, respectively.

Thymic development of Lck-Cre-TCTPf/d and CD4-Cre-TCTPf/d mice

All DN thymocyte subsets, i.e., DN1 (Lin–, CD25–, CD44–), DN2 (Lin+CD25–, CD44+), DN3 (Lin+CD25+, CD44–) and DN4 (Lin+CD25+, CD44+) express TCTP (Fig. 1A, bottom panel). Western blotting analysis (Fig. 1A, bottom panel) revealed that the TCTP protein levels in thymocytes from Lck-Cre-TCTPf/d mice (hereafter referred to as Lck-TCTPf/d mice) at both DN1 and DN2 stages were reduced to ~40% of that in their control counterparts, i.e., cells from Lck-Cre-TCTPf/f mice (hereafter, unless otherwise indicated all control mice refer to this genotype). A significantly more reduction (retaining ~24% of control cell levels) was observed at the DN3 stage. At the DN4 stage nearly no TCTP protein could be detected (retaining ~8% of control cell levels). Similar extent of reduction was also observed at the mRNA levels (data not shown). Total thymic cellularity of such mutant mice was reduced by approximately 25%, compared with littermate controls. Similar extent of reduction was also observed for DP thymocytes in these mutant mice, suggesting a partial block in the DN to DP transition or a reduction in cell survival. To examine this directly, we analyzed the numbers of DN subpopulations. As shown in Fig. 1C, the number of DN3 thymocytes was slightly increased (~30%) in Lck-TCTPf/d mice, suggesting a partial block in the DN3 to DN4 maturation step.

In contrast, those thymocytes in Lck-TCTPf/d mice that did develop into the DP or SP stage did not have any detectable level of TCTP (Fig. 1A), suggesting that these cells were not those that had escaped the Cre-mediated deletion events. Instead, it suggests that in the absence of TCTP, DP thymocytes can still initiate positive selection and develop into either the CD4 or CD8 SP stage. Flow cytometric analysis confirmed this notion, as similar percentages of cells expressing high levels of molecules indicative of cells undergoing positive selection such as CD69, TCRβ and CD5 were
FIGURE 1. Loss of TCTP during early thymocyte development resulted in a partial DN3 block and attenuated maturation of SP thymocytes. A, Western blotting analysis of TCTP protein from sorted subsets of thymocytes as indicated from Lck-Cre-TCTPf/−/H11002 (control or C) or Lck-Cre-TCTPf/d (Lck-TCTPf/K) mice. Numbers on the bottom of the lower panel are relative TCTP protein levels (normalized to β-actin levels) in each DN subsets as indicated (all TCTP levels in control counterparts were set as 100). B, top panel, Flow cytometric analyses of CD4 and CD8 expression on thymocytes from mice as indicated. (lower panel) Lineage negative (Lin−; CD4− CD8− B220− CD3− NK1.1− TER119− Gr-1− CD11b−) DN cells were stained for CD25 and CD44 and analyzed by flow cytometry. Numbers indicate percentage of cells in each marked gate. C, Graphical representation of total, DN, DP, CD4SP(4SP) and CD8SPTCRβhigh (8SP) thymocyte and DN subset numbers. Subsets are classified as CD44−CD25− (DN1), CD44−CD25+ (DN2), CD44+CD25− (DN3) and CD44+CD25+ (DN4). Five to seven control or Lck-TCTPf/K mice were analyzed. Cell numbers were calculated from populations gated as shown in B. D, Thymocytes from the indicated mice were stained for CD4, CD8, TCRβ, and one of the following maturation markers (CD69, CD5, HSA or IL-7Rα) or isotype control Ab for IL-7Rα. Histograms show TCRβ, CD69, CD5, HSA or IL-7Rα levels on gated DP and mature (TCRβhigh) CD4SP or CD8SP thymocytes. Numbers are percentages of cells in the indicated populations. Data are representative of at least three experiments.
observed on both control and Lck-TCTP−/− DP cells (Fig. 1D). In contrast, although Lck-TCTP−/− DP cells could initiate positive selection, maturation of the resultant SP cells appeared to be partially delayed or impaired, because the percentage of SP cells that completed positive selection, i.e., the TCR selection, maturation of the resultant SP cells appeared to be partial or slightly increased, a markedly reduced level of the CD8+ T cells was found both in the LN and spleens of these mice, whereas the number of their CD4+ T cells was only reduced in the spleen but not in the LN (Fig. 3, C and D). Western blotting analysis revealed that all T cells present in the peripheral lymphoid organs of both Lck-TCTP−/− and CD4-TCTP−/− mice expressed no or extremely low levels of TCTP, suggesting that most of these T cells were not those that had escaped from the Cre-mediated deletion events (Fig. 3E).

We next examined whether TCTP-deficient T cells were more susceptible to cell death than control cells. To address this issue, the in vitro “die of neglect” assay was first conducted. As shown in Fig. 4, a slight increase of apoptosis (~5–10%) was consistently observed in freshly isolated (i.e., at the zero time point) mature TCTP-deficient CD4+ and CD8+ T cells, compared with their control counterparts. As they were cultured in the simple growth medium supplemented with 10% serum, both TCTP-deficient CD4+ and CD8+ T cells still consistently died faster than their control counterparts (Fig. 4 and data not shown for cells from Lck-TCTP−/− mice). Next, we examined whether mature T cells generated in the thymus manifested a similar property in vivo. To address this issue, both CD4SP and CD8SP (TCRβhigh) cells purified from the thymus of control or Lck-TCTP−/− mice were labeled with CFSE and injected into the tail vein of the control mice. Three days after injection, the number of CFSE-labeled cells present in the peripheral lymphoid organs of the recipient mice was analyzed. As shown in Table I, compared with their control counterparts (taken as 100%), approximately 63% of TCTP−/− CD4SP and 45% of TCTP−/− CD8SP cells were detected in LN of recipient mice, whereas approximately 80% and 71% of TCTP−/− CD4SP and CD8SP cells, respectively, was detected in the spleen of recipients. Considering that TCTP−/− CD4SP and CD8SP cells both homed to the spleen slightly better than their control counterparts and that their homing to LN was only minimally reduced (Table II), the results shown in Table I suggest that TCTP-deficient mature T cells lose viability faster than control cells in this in vivo system. Taken together, both assay systems suggest that TCTP plays an important role in the viability control of mature T cells.

**FIGURE 2.** Modest increase of mature CD8SP thymocytes in CD4-Cre-TCTP+/− (CD4-Cre-TCTP−/−) mice. A, Western blotting analysis of TCTP protein from sorted subsets of thymocytes from CD4-Cre-TCTP+/− (control) or CD4-Cre-TCTP−/− mice. B, top panel, Expression of CD4 and CD8 on thymocytes from mice as indicated. Numbers indicate percentage of cells in each marked gate. Bottom panel, Graphical representation of total, DN, DP, mature (TCRβhigh) CD4SP (4SP) and CD8SP (8SP) thymocyte numbers (n = 6).

**CRITICAL FUNCTIONS OF TCTP IN THE T CELL LINEAGE**

In contrast to the slight reduction (~30%) of both CD4SP and CD8SP cells in the thymus of Lck-TCTP−/− mice (Fig. 1C), a significant reduction of mature CD4+ and CD8+ T cells (>50%) was observed both in the lymph nodes (LN) and spleens of these mutant mice (Fig. 3, A and B). Interestingly, in CD4-TCTP−/− mice, even though the numbers of the thymic CD4 or CD8 SP cells appeared to be normal or slightly increased, a markedly reduced level of the CD8+ T cells was only reduced in the spleen but not in the LN (Fig. 3, C and D). Western blotting analysis revealed that all T cells present in the peripheral lymphoid organs of both Lck-TCTP−/− and CD4-TCTP−/− mice expressed no or extremely low levels of TCTP, suggesting that most of these T cells were not those that had escaped from the Cre-mediated deletion events (Fig. 3E).

Enforced expression of Bcl-2 rescued the partial block of early thymocyte development but not peripheral T cell lymphopenia of Lck-TCTP−/− mice

Bcl-2 plays an important role in T cell development both at the DN and SP stages (20–23). Next, we examined whether enforced expression of Bcl-2 could rescue the defects observed in Lck-TCTP−/− mice. To address this issue, we bred the Eκ-bcl-2 transgene into control or Lck-TCTP−/− mice (Fig. 5A). As shown in Fig. 5B, Lck-TCTP−/− mice carrying the Eκ-bcl-2 transgene (Lck-TCTP−/− Bcl-2F8) manifested similar numbers of total thymocytes and DP cells as control mice with (control Bcl-2F8) or without overexpression of Bcl-2, suggesting that enforced expression of Bcl-2 rescued the partial DN3 block of TCTP-deficient thymocytes. Moreover, transgenic over-expression of Bcl-2 also due to a feedback loop regulation resulting from the defects in the peripheral lymphoid tissues of these mutant mice (see below).

Reduced survival of TCTP-deficient T cells

In contrast to the slight reduction (~30%) of both CD4SP and CD8SP cells in the thymus of Lck-TCTP−/− mice (Fig. 1C), a significant reduction of mature CD4+ and CD8+ T cells (>50%) was observed both in the lymph nodes (LN) and spleens of these mutant mice (Fig. 3, A and B). Interestingly, in CD4-TCTP−/− mice, even though the numbers of the thymic CD4 or CD8 SP cells appeared to be normal or slightly increased, a markedly reduced level of the CD8+ T cells was only reduced in the spleen but not in the LN (Fig. 3, C and D). Western blotting analysis revealed that all T cells present in the peripheral lymphoid organs of both Lck-TCTP−/− and CD4-TCTP−/− mice expressed no or extremely low levels of TCTP, suggesting that most of these T cells were not those that had escaped from the Cre-mediated deletion events (Fig. 3E).
FIGURE 3. Peripheral T cell lymphopenia of TCTP-deficient mice. Profiles of CD4 and CD8 staining on lymph node (LN) (A) and spleen (B) cells from Lck-Cre-TCTP<sup>−/−</sup> (Lck-control) and Lck-TCTP<sup>−/−</sup> mice. Bar graphs in lower panel of (A and B) show total cellularity and absolute numbers of T (CD4<sup>+</sup> or CD8<sup>+</sup>) or B (B220<sup>+</sup>) cell subset in LN (A) or spleen (B) from 8-wk-old control or Lck-TCTP<sup>−/−</sup> mice. Data are presented as mean ± s.e.m. (n = 8). C and D, Same analysis as that shown in (A and B) except that CD4-Cre-TCTP<sup>−/−</sup> (CD4-control) and CD4-TCTP<sup>−/−</sup> mice were analyzed. (n = 6). E, Western blotting analysis of total cell lysates prepared from CD3<sup>+</sup> (left), CD4<sup>+</sup> or CD8<sup>+</sup> (right) T cells sorted from spleens or lymph nodes of the indicated mice.
significantly restored the numbers of both CD4SP and CD8SP thymocytes in Lck-TCTP−/− mice (Fig. 5B).

We next examined whether enforced expression of Bcl-2 could rescue peripheral T cell lymphopenia of Lck-TCTP−/− mice. In this experiment, we noticed that enforced expression of Bcl-2 manifested a minor phenotype by itself, i.e., it resulted in a slight increase (1/2 to 1-fold) of both CD4+ and CD8+ T cells in the spleen of control mice (Fig. 5, C and D). Similar effect was also observed when Bcl-2 was over-expressed in Lck-TCTP−/− mice. However, the restored CD4+ or CD8+ number was still significantly less than that in the control mice, and was much lower than the CD4+ or CD8+ number in control mice expressing the Eμ-bcl-2 transgene (Fig. 5D), indicating that enforced expression of Bcl-2 could not rescue peripheral T cell lymphopenia of Lck-

![FIGURE 4. TCTP played a role in T cell survival. Splenocytes from control or CD4-TCTP−/− mice were cultured in medium supplemented with 10% serum. At various times after culturing, apoptotic cell percent-

age were quantified by annexin V staining assay. Shown here is one representative from four independent experiments with very similar results. For each independent experiment, splenocytes from one to three pairs of age- and gender-matched control and TCTP−/− (Lck-TCTP−/− or CD4-TCTP−/−) mice were analyzed. Data presented (means ± SE) here are from one representative experiment where cells from three pairs of mice (control and TCTP−/−) were analyzed at the same time.

TCTP−/− mice. All together, these results suggest that TCTP regulates early thymocyte development likely by modulating the expression of Bcl-2 or its functional homolog. However, a much more complicated role that TCTP may play in regulating T cell homeostasis in the peripheral tissues.

**TCTP-deficient T cells manifested defects in TCR-induced proliferation**

TCTP expression was prominently induced in T cells following TCR stimulation (13) (Fig. 6E). To study the role of TCTP in this process, naïve lymph node T cells (CD4+CD25+CD44low or CD8+CD44low) were sorted from control or CD4-TCTP−/− mice (or Lck-TCTP−/− mice as indicated. Note that TCTP−/− T cells from these two mutant mice behaved quite similar) and activated with plate-bound anti-TCRβ and anti-CD28. As shown in Fig. 6A, proliferation of TCTP−/− CD4+ T cells, measured by the 3H-incorporation experiment, was severely impaired compared with that of control cells. Similar results were observed for TCTP−/− CD8+ T cells (Fig. 6A). Flow cytometric analysis of cell size and DNA content further showed that, compared with control cells, much fewer TCTP−/− T cells had blasted and entered into S phase 24 h post-TCR stimulation (Fig. 6B). Next, the CFSE dilution method was used to monitor T cell division in response to anti-

TCRβ/anti-CD28 stimulation. As shown in Fig. 6C, proliferation of TCTP−/− CD4+ T cells, under the same stimulation conditions, TCTP−/− T cells consistently made less cell division than control T cells. Western blotting analysis revealed that a markedly delayed and/or reduced induction of cyclins known to play important roles in G1-S progression of cell cycle such as cyclins E2 and D3 was observed in TCR-activated TCTP−/− T cells (Fig. 6E). Taken together, these results suggest that overall reduction in proliferation of TCTP-deficient CD4+ and CD8+ T cells was likely due to a severe delay in initial cell-cycle entry following TCR stimulation.
Discussion
In this study, by conditional knockout approach, we explored the function of TCTP in the T cell lineage. We demonstrate that, although TCTP is expressed in every thymocyte subset, conditional deletion of TCTP before the β-section checkpoint only results in a slight defect in thymocyte development with a partial block at the

**FIGURE 5.** Enforced expression of Bcl-2 rescued the partial block of thymocyte development, but not peripheral T cell lymphopenia of Lck-TCTP^{−/−} mice. A, Western blotting analysis of Bcl-2 expression in thymocytes from control or Lck-TCTP^{−/−} mice carrying or not carrying the Eμ-Bcl-2 transgene (Bcl-2{Tg}). B, Total thymic cellularity and absolute numbers of DP and mature (TCRβ^{high}) CD4SP or CD8SP thymocytes from mice as indicated. C, Representative profiles of CD4 and CD8 staining on splenocytes from mice with the indicated genotype. D, Total splenocyte cellularity and absolute numbers of splenic CD4^{+} or CD8^{+} T cells in the same mice shown in B.
The modest reduction of both DP and SP thymocytes correlated with the partial DN3 block observed in Lck-TCTP−/− thymus. However, the exact mechanism of such partial DN3 block is not clear at this point. The known functions of TCTP in the modulation of apoptosis (11–13), as well as our observation that the expression of a Bcl-2 transgene restores both DP and SP cell numbers in Lck-TCTP−/− mice, implicate that the survival function of TCTP plays a role in thymocyte development. However, such interpretation seems to contradict the fact that TCTP deficiency results in a modest increase of DN3 thymocytes, a result actually suggesting that a slight block in thymocyte progression into the DP stage has occurred. In contrast, it was previously shown that thymocyte differentiation could continue spontaneously in vitro when cell viability was sustained by constitutive expression of Bcl-2 (16). We thus propose that, although TCTP plays a modest role in thymocyte development, such a role appears to be related, at least partially, to its survival functions.

In contrast to the very modest phenotype observed in the thymus of Lck-TCTP−/− and CD4-TCTP−/− mice, both mutant mice manifested a significant reduction of mature T cells in their peripheral lymphoid tissues. Detailed analysis revealed that such phenotype is mainly due to a decreased viability of mature TCTP-deficient T cells. Surprisingly, enforced expression of Bcl-2 could not rescue peripheral T cell lymphopenia of Lck-TCTP−/− mice, even though such treatment efficiently rescued the modest defect of thymocyte development in these mutant mice. This result suggests that TCTP may regulate thymocyte development and peripheral T cell homeostasis via distinct signaling networks. For the former process, TCTP may modulate the activity of a pathway that leads to the activation of Bcl-2 or its functional homologue. One such candidate is the IL-7 signaling pathway, as this pathway plays a pivotal role in the expansion of DN thymic precursors (24) and can activate the expression of Bcl-2 (20). For peripheral T cell homeostasis, TCTP may activate another signaling pathway that can modulate the expression or activity of a different set of protein(s) or additional factor(s) other than Bcl-2. One candidate protein that TCTP may regulate to promote T cell survival is Mcl-1, as Mcl-1 is known to be a viability factor for T cells (25) and TCTP can interact with Mcl-1 and enhance Mcl-1’s protein stability and anti-apoptotic activity (12). However, preliminary results show that Mcl-1 protein levels are not altered in TCTP-deficient thymocytes.
or mature T cells (supplemental Fig. S1). More experiments will be required to address how TCTP regulates peripheral T cell homeostasis.

On the other hand, how can TCTP regulate thymocyte development and peripheral T cell homeostasis via distinct signaling networks during different developmental stages of the T cell lineage? Given that TCTP was implicated to have a role in protein translation (9, 10) and gene transcription (15), one likely scenario is that TCTP regulates the expression and/or modulates the activity of a distinct set of proteins in the T cell lineage in a stage-specific manner.

In addition to regulating thymocyte development and peripheral T cell homeostasis, TCTP was found to play an important role in T cell proliferation. Upon T cell activation by TCR ligation and CD28 costimulation, TCTP was prominently induced (13). We demonstrate that such induction is critical for TCR-activated T cells to enter S phase at a proper time. In the absence of TCTP, the proliferation of TCR-stimulated T cell was significantly impaired, a result mainly due to a severe delay in initial cell cycle entry. Given that TCTP acts as the GEF of Rheb that controls TOR (target of rapamycin) activity in Drosophila (2) and that mTORC1 is known to regulate TOR-induced T cell proliferation (26), we were curious whether TCTP would regulate the activation of the mTORC1 pathway in T cells following TCR stimulation. Preliminary results suggest that this was not the case, as mTORC1-mediated phosphorylation of p70-S6K at Thr389 remained normal in TCR-activated TCTP−/− T cells (data not shown). More experiments will be required to address the signaling pathway involved in TCTP-regulated T cell proliferation.

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Disclosures

The authors have no financial conflict of interest.

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