Transitionally controlled tumor protein (TCTP) downregulates Oct4 expression in mouse pluripotent cells

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The present study aimed to investigate the function of translationally controlled tumor protein (TCTP) in the regulation of Oct4 in mouse embryonic carcinoma P19 cells and mouse J1 embryonic stem (ES) cells. The mRNA level of endogenous TCTP in somatic cells was 2-4 folds higher than that in pluripotent P19 and J1 ES cells. Overexpression of TCTP in mouse pluripotent cells not only reduced the level of Oct4 transcription, but also decreased the pluripotency of stem cells. The N-terminal end of TCTP (amino acids 1-60) played an important role in suppressing the Oct4 promoter. Moreover, overexpression of TCTP in P19 cells suppressed the Oct4 promoter activity in a dose- and a time-dependent manner. In addition, knockdown of TCTP by small interfering RNA increased the expression of Oct4. Our study indicates that TCTP downregulates the Oct4 expression by binding the Sf1 site of Oct4 promoter in mouse pluripotent cells. [BMB reports 2012; 45(1): 20-25]

INTRODUCTION

Octamer binding transcription factor 4 (Oct4), a member of the POU family, is an essential factor for maintaining the pluripotency and self-renewal of embryonic stem (ES) cells. Oct4 is also the core transcription factor being used to induce somatic cells into the induced pluripotent stem (iPS) cells (1). Oct4 is POU family, is an essential factor for maintaining the pluripotency of mouse embryonic stem (ES) cells. The mRNA level of endogenous TCTP in somatic cells was 2-4 folds higher than that in pluripotent P19 and J1 ES cells. Overexpression of TCTP in mouse pluripotent cells not only reduced the level of Oct4 transcription, but also decreased the pluripotency of stem cells. The N-terminal end of TCTP (amino acids 1-60) played an important role in suppressing the Oct4 promoter. Moreover, overexpression of TCTP in P19 cells suppressed the Oct4 promoter activity in a dose- and a time-dependent manner. In addition, knockdown of TCTP by small interfering RNA increased the expression of Oct4. Our study indicates that TCTP downregulates the Oct4 expression by binding the Sf1 site of Oct4 promoter in mouse pluripotent cells.

RESULTS

TCTP expression and subcellular localization
To verify the expression of constructs, protein extracts from P19 cells were prepared for western blot analysis at 48 h after transfection. A 45 kDa band representing the EGFP-TCTP fusion protein was observed in pEGFP-TCTP (AA.1-172) transfected cells, while a 27 kDa EGFP band was detected in cells transfected with pEGFP-C1 (Fig. 1A). In addition, the EGFP-TCTP (AA.1-172) fusion protein was distributed both in the nucleus and in the cytoplasm of P19 and NIH3T3 cells (Fig. 1B).

TCTP overexpression suppresses Oct4 gene expression
To investigate the level of endogenous TCTP expression, we
compared the TCTP expression pattern in P19, C2C12, NIH3T3 and J1 ES cells by real time RT-PCR. The TCTP mRNA was detected in four cell lines (Fig. 2A). Moreover, the mRNA level of TCTP was 2-4 folds lower in pluripotent cells (P19 and J1 ES) than that in somatic cell lines (NIH3T3 and C2C12). To evaluate whether TCTP regulated Oct4 expression, the pEGFP-TCTP (AA.1-172) was transfected into P19 and J1 ES cells, respectively. In (+TCTP) P19 cells transfected with pEGFP-TCTP (AA.1-172), the mRNA level of TCTP was significantly increased and the level of Oct4 was significantly decreased compared to the control (-TCTP) cells transfected with pEGFP-C1 (Fig. 2B). Similar results were observed in J1 ES cells (Fig. 2C).

Since the level of Oct4 expression is crucial to retaining the pluripotency of ES cells, we investigate whether the down-regulation of Oct4 expression due to overexpressing TCTP could induce ES cells differentiation. The differentiation of ES cells was evidenced by the loss of typical ES morphology and reduced alkaline phosphatase (AP) activity in J1 ES cells at 72 h after TCTP transfection (Fig. 2D).

To further evaluate the effect of TCTP on Oct4 expression, we did the luciferase assay by cotransfection of pOct4-Luc with either pEGFP-TCTP (AA.1-172) or TCTP truncated constructs into P19 cells. The Oct4 promoter activity in control cells transfected with pEGFP-TCTP (AA.1-172) was 29 percent (P < 0.05) of that in control cells transfected with pEGFP-C1. In addition, overexpression of TCTP truncated construct pEGFP-TCTP (AA.1-60) reduced the Oct4 promoter activity to 27 percent (P < 0.05) and pEGFP-TCTP (AA.61-172) 50 percent (P > 0.05) of the control cells (Fig. 3A).

The previous study reported TCTP interacted with Oct4 by binding to the Sf1 site of the Oct4 promoter (14). To directly address the role of TCTP in Oct4 promoter regulation, TCTP was cotransfected with either the Oct4 report plasmid with Sf1 site or the plasmid without Sf1 site into P19 cells. Upon transfection into P19 cells, the Sf1 site deletion prevented the suppression by TCTP (Fig. 3B). In addition, TCTP repressed the Oct4 promoter activity both in a time-dependent manner (Fig. 3C) and in a dose-dependent manner (Fig. 3D). These observations further demonstrated that TCTP negatively regulated the expression of Oct4.

Knockdown of TCTP by siRNA upregulates the Oct4 transcription

In light of the above observations that the exogenous TCTP re-
TCTP represses Oct4 expression in mice
Xiang Cheng, et al.

Fig. 4. siRNA decreases TCTP mRNA expression and increases the Oct4 transcription. The siRNAs against TCTP were synthesized, including T-90, T-236, T-408 and negative control (NC). Cells were transiently transfected with different siRNAs for 48 h, and the inhibition efficiency was determined by real time RT-PCR. (A) Knockdown of TCTP in P19 cells. Control, untransfected P19 cells. (B and C) siRNA T-90 affects the expression of TCTP and Oct4 in P19 cells and J1 ES cells. Control, cells transfected with negative control siRNA. *P < 0.05; **P < 0.01.

Discussion

Oct4 plays a crucial role in the development and is an essential factor to maintain the pluripotency of the ES cells. Oct4 expression was regulated by cis-regulatory elements including three important elements: the distal enhancer, the proximal enhancer and the proximal promoter (16). Oct4 and Sox2 activated the Oct4 transcription by binding an Oct4/Sox2 element (17). The caudal-type homeobox transcription factor 2 (Cdx2) suppressed Oct4 gene by binding to the distal enhancer (18). The orphan nuclear receptor liver receptor homolog 1 (LRH1) was a positive regulator of Oct4 by binding to the proximal enhancer and proximal promoter (11). The precise level of Oct4 was regulated by the balance between these positive and negative regulators (19). In this study we identified TCTP as a negative regulator of Oct4 by binding the Sf1 site within the proximal promoter in mouse pluripotent cells.

TCTP is highly conserved among eukaryotic organisms, indicating that it plays an essential role in the normal development (8). TCTP has a growth related function as the overexpression or knockdown TCTP disturbed the cell growth.
The physiological role of TCTP epitomized that in TCTP knockout mice homozygous mutants (TCTP−/−) were embryonic lethal and the knockout embryos suffered a high incidence of apoptosis (22). In Xenopus oocyte, it has been confirmed that TCTP has a role in transcriptional regulation of Oct4 by directly binding to the Sf1 region, which is highly conserved between mouse and Xenopus, of Oct4 promoter (14).

The investigation of the TCTP subcellular localization in P19 and NIH3T3 cells indicated that TCTP was located not only in the nucleus but also in the cytoplasm (Fig. 1B), which was consistent with the recent report revealing its distribution in mouse ES cells and embryonic carcinoma cells (20). In addition, the downregulation of Oct4 by TCTP was confirmed through the luciferase assay by co-transfecting the EGF-P-TCTP fusion constructs with the Oct4 promoter reporter plasmid pOct4-luc. Of note, the TCTP truncated construct pEGFP-TCTP (AA.1-60) containing the TCTP1 motif had a similar efficiency with the pEGFP-TCTP (AA.1-172) of repressing the Oct4 promoter activity, which suggested that this region might contain the motif bound to the Oct4 promoter. Furthermore, the effect of exogenous TCTP downregulating Oct4 promoter activity represented a time-dependent and a dose-dependent manner in P19 cells. This regulation was due to the TCTP interacting with Oct4 promoter by binding the Sf1 site (Fig. 3B), which was consistent with the previous report (14). Moreover, knockdown of TCTP by small interfering RNA upregulated Oct4 transcription in both P19 and J1 ES cells (Fig. 4), further confirming that the TCTP gene downregulated the Oct4 expression.

Our result that TCTP was a negative regulator of Oct4 in mouse pluripotent cells was consistent with the recent reports which showed that TCTP interacted with nucleophosmin to form the complex to play the role during mitosis in mouse ES cells (20), and the knockdown of TCTP induced Oct4 expression in mouse ES cells (15). However, our data was conflicting with the report which showed TCTP activated Oct4 in Xenopus oocyte (14). One explanation is that amphibian oocytes and mammalian cells may have different epigenetic modification manners on Oct4 regulation through TCTP, such as DNA methylation (14) and protein phosphorylation (23).

In summary, in this study we identified that the subcellular localization of the TCTP was present both in the nucleus and in the cytoplasm in P19 and NIH3T3 cells. Overexpression of TCTP gene decreased the Oct4 expression, and the knockdown of TCTP by small interfering RNA molecules increased Oct4 expression in mouse P19 and J1 ES cells. Our observation indicates that TCTP is a negative regulator of the Oct4 gene by binding the Sf1 site in mouse pluripotent cells.

MATERIALS AND METHODS

Cell culture
The mouse embryonic carcinoma cell line P19 was cultured in α-MEM (Invitrogen) supplemented with 10% fetal bovine se-

run (HyClone, USA). The mouse ES cell line J1 was maintained on the feeder layer of mouse embryonic fibroblasts in ES cell media [Dulbecco’s modified eagle’s medium (high glucose, USA) supplemented with 15% fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, and 1,000 U/ml leukemia inhibitory factor (LIF, Gbico)]. The mouse C2C12 myoblasts and NIH3T3 fibroblasts were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum.

Vector constructions
The TCTP cDNA was amplified from the total RNA extracted from the P19 cells using the primers listed in the Table S1. PCR products were ligated into a pGEM-T Easy vector for sequencing. The TCTP coding sequence was subcloned into pEGFP-C1 to construct the pEGFP-TCTP (AA.1-172) vector. The pEGFP-TCTP (AA.1-172) plasmid was digested with KpnI site and the two fragments including TCTP (AA.1-60) fused with EGFP and TCTP (AA.61-172) were purified. The pEGFP-TCTP (AA.1-60) was constructed with self-ligation of the KpnI-digested pEGFP-TCTP (AA.1-172) plasmid. The TCTP fragment (AA.61-172) was ligated to KpnI digested pEGFP-C1 to construct the pEGFP-TCTP (AA.61-172). The mouse Oct4 promoter region (−682 to +112), the region (−136 to +112) and the region (−86 to +112) were amplified from mouse liver genomic DNA and was ligated into the pGEM-T Easy vector. The mouse Oct4 promoters were subcloned into the vector pGL3-basic and positive clones were confirmed by sequencing. The mouse Oct4 reporter vector containing the region (−682 to +112) was named as pOct4-Luc.

Quantitative real time RT-PCR
Total RNA was isolated with Trizol reagent (Invitrogen, USA) and reverse transcribed with RevertAid first-strand cDNA synthesis kit (Fermentas, Canada). Relative mRNA levels were evaluated by real-time RT-PCR carried out by using the SYBR Premix Ex Taq™ kit (TakaRa, Japan). The β-actin was used as the internal control. Sequences of primers are listed in Table S1. All reactions were performed in triplicate, and the data were the average of three independent experiments.

Western blot and alkaline phosphatase assay
The plasmids, pEGFP-TCTP (AA.1-172) and pEGFP-C1, were transfected into P19 cells, respectively. At 48 h after the transfection, total protein was extracted from each sample. The equal amount of protein samples were separated on 12% SDS-PAGE gel and then transferred to the nitrocellulose membrane. The membrane was blocked by 5% skim milk, and then incubated with anti-GFP antibody (1 : 2,000, Abcam, USA), and followed by incubation with HRP-conjugated secondary antibody. Immunoreactive bands were detected by ECL kit (Pierce, USA). Alkaline phosphatase assay was performed based on the manufacturer’s instruction (Sigma).
Transient transfection and luciferase assay
P19 cells and J1 ES cells were transfected with the different constructs using the Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen). Due to the low efficiency of transfection, J1 ES cells were transfected following the protocol described in the recent report (24). Cells were harvested at various time points after transfection, and luciferase activity was determined with the Enhanced Luciferase Assay Kit (BD Bioscience, USA) using the Centro LB960 96-well luminometer (Berthold Technologies).

siRNA interference
Based on mouse TCTP cDNA sequence, three siRNAs (T-90, T-236, T-408) of TCTP gene and a negative control (NC) siRNA were synthesized. The detailed information of siRNA sequences is listed in Table S2. P19 cells and J1 ES cells were transfected with 50 nM of experimental siRNAs and control siRNA using the Lipofectamine 2000 reagent (Invitrogen). At 48 h after transfection, total RNA were isolated for real time RT-PCR assay described in previous section to determine the mRNA expression of TCTP and Oct4 genes.

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REFERENCES
reveals that TCTP functions as an essential factor for cell proliferation and survival in a tissue- or cell type-specific manner. 

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