TCTP Overexpression Associates with the Tumorigenesis in Opisthorchiasis-Associated Cholangiocarcinoma

Nattaporn Phanthaphol* Dr.Anchalee Techasen **Dr.Watcharin Loilome***

Dr.Puangrat Yongvanit**** Dr.Nisana Namwat*****

ABSTRACT

The translationally controlled tumor protein (TCTP) overexpression was founded in many cancers indicating its role in tumorigenesis. We aimed to investigate the expression profile of TCTP using immunohistochemistry during carcinogenesis of cholangiocarcinoma (CCA) that was associated with liver fluke (*Opisthorchis viverrini;* Ov) infection in hamster model. We found that TCTP protein was strongly expressed in biliary ducts along CCA carcinogenesis whereas no expression was observed in untreated control group. Besides the roles in tumorigenesis, we also investigated the roles of TCTP in CCA progression using CCA cells. The depletion of TCTP significantly decreased a transcription repressor SNAIL *(***$P<0.001$*)* in KKU-M055 and KKU-M214, suggesting its role in epithelium-mesenchymal transition (EMT). Nevertheless, the downstream targets of SNAIL, E-cadherin (E-cad) and N-cadherin (N-cad) were slightly altered upon TCTP depletion. In summary, TCTP might plays a role in the CCA carcinogenesis which is related to Ov infection and contributes metastasis. Therefore, further experiments need to be performed. The finding might be useful to target TCTP for CCA prevention and therapy.

Keywords: TCTP, Cholangiocarcinoma, EMT, SNAIL

---

* *Student, Master of Science Program in Medical Biochemistry and Molecular Biology, Department of Biochemistry, Faculty of Medicine, Khon Kaen University*

** *Lecturer, Center for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Science, Khon Kaen University*

*** *Assistant Professor, Department of Biochemistry, Faculty of Medicine, Khon Kaen University*

**** *Professor, Department of Biochemistry, Faculty of Medicine, Khon Kaen University*

***** *Associate Professor, Department of Biochemistry, Faculty of Medicine, Khon Kaen University*

Introduction

Cholangiocarcinoma (CCA) refers to cancer of epithelial cells lining the bile duct. It is a major public health problem in the Northeast of Thailand. The epidemiological and experimental evidences indicate that the infection with *Opisthorchis viverrini* (Ov) is a major risk factor for CCA in this area (Sripa and Pairojkul, 2008). The CCA progression is relatively slow and patients come to the hospital mostly with the late stage. Chemotherapy combined with surgery can make a tumor collapsed and prolong the patients' survival rather than surgery alone (Valle *et al*., 2009). Therefore, studying in mechanisms especially the changes in molecular pathways driving CCA carcinogenesis and progression needs to be investigated. The translationally controlled tumor protein (TCTP) was recognized as tumor-associated protein and its overexpression protects cancer cells from oxidative stress induced cell death (Lucibello *et al*., 2011) and also induces epithelial-mesenchymal transition (EMT) to promotes cell migration, invasion and metastasis (Bae *et al*., 2015). From these evidences, we hypothesized that TCTP plays roles in CCA carcinogenesis which is related to Ov infection and contributes to CCA progression by regulating EMT.

Objective of the study

We aimed to investigate the expression pattern of TCTP along Ov-induced hamster CCA tissues. Moreover, we investigated a possible association of TCTP and EMT marker, SNAIL, E-cadherin (E-cad) and N-cadherin (N-cad) in CCA cells.

Methodology

Ov-associated CCA hamster tissues

The protocol of animal induction model was approved by the Animal Ethics Committee of the Faculty of Medicine, Khon Kaen University, Thailand (#AE002/2002). Animals were induced by Ov metacercariae infection combined with *N*-nitroso-dimethylamine (NDMA) and paraffin embedded hamster liver tissue slides were archived difference time points, as previously described (Loilome *et al.*, 2006).

Cell culture

Human CCA cell lines (KKU-M055 and KKU-M214) were obtained from the specimen bank of the Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand. All cell lines were maintained in Ham's F-12 medium supplemented with 2 mg/ml $NaHCO_3$, 100 U/ml penicillin, 100 mg/ml streptomycin and 10 % fetal bovine serum at 37°C with 5 % CO_2.

Transient knockdown of TCTP by small interfering RNA (siRNA)

The siRNAs of human TCTP (SMART pool ON-TARGET plus siRNA, ID L-004559-00-0005) and control-siRNA (ID D-001810-10-05) were purchased from Dharmacon Inc. (Lafayette, CO, USA). CCA cells (6×10^4) were plated into a 6-well plate and incubated overnight before the beginning of transfection. The siRNAs and lipofectamine RNA iMAX reagents were complexed in Opti-MEM® I medium (Invitrogen, USA), as a final concentration of 50 μM. The siRNA-treated cells were further maintained for 72 h to achieve a complete transfection. The successful suppression of TCTP expression was verified by the western blot analysis.

Western blotting

Cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed with cell lysis buffer.

The protein concentration in the solution fractions were determined by Pierce BCA™ Protein Assay Kit. Equal amounts of protein were resolved on 10% SDS-PAGE and transferred to PVDF membrane. After blocking the non-specific sites with 5% (w/v) skim milk solution, membrane was probed with primary antibodies against TCTP, N-cad (Abcam), E-cad (BD Biosciences), SNAIL (Santa Cruz Biotechnologies) and ß-actin (Sigma-Aldrich). The blots were rinsed three times and subsequently incubated with a secondary antibody conjugated with horseradish peroxidase. Immunodetection was performed using the enhanced chemi-luminescence system (Amersham).

Statistical analysis

The statistical significance of different data was determined by the Student's *t* test. A P value of less than 0.05 was statistically significant.

Results

TCTP expression in hamster liver tissues during CCA development

The expression profile of TCTP proteins during the carcinogenesis in the Ov plus NDMA induced CCA in hamster model was investigated. TCTP staining was negative in the untreated group (Fig. 1A) and the normal bile duct in Ov plus NDMA group at 14 days post infection (p.i.) (Fig. 1B). Intense TCTP staining was found in cytoplasm of bile duct epithelia in Ov plus NDMA group at 30 days and 60 days p.i. (Fig. 1C and 1D) when hyperplastic duct has developed. More intense staining of TCTP was observed at 120 days p.i. when the biliary ducts appeared as dysplastic lesion (Fig. 1E) and 180 days p.i. when CCA was fully developed (Fig. 1F).

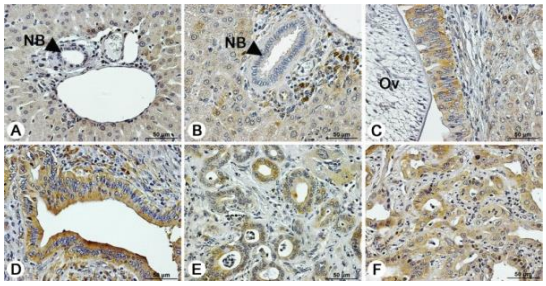

Figure 1 TCTP expression in Ov associated cholangiocarcinogenesis. (A) Untreated; (B) Ov plus NDMA at 14 days p.i.; (C) Ov plus NDMA at 30 days p.i.; (D) Ov plus NDMA at 60 days p.i.; (e) Ov plus NDMA at 120 days p.i.; (F) Ov plus NDMA at 180 days p.i.. *NB* normal bile duct. Images were taken at 400x. Scale bar 50 µm.

Downregulating of TCTP altered EMT marker

Immunoblotting demonstrated the alteration of EMT markers in siTCTP transfected CCA cells, KKU-M055 and KKU-M214. The level of SNAIL was significantly reduced whereas the epithelial marker E-cad and mesenchymal N-cad were unchanged (Fig. 2).

Discussion and Conclusion

This study aimed to reveal TCTP expression in Ov-induced CCA genesis and to investigate the possible association of TCTP and EMT along CCA progression. The TCTP immunostaining of liver tissues from Ov induced CCA hamster addressed that TCTP was switched on the cytoplasm of bile duct cells at 30 days post infection and gradually increased along the carcinogenesis (Fig. 1C-1F). In contrast, TCTP expression was not detectable (Fig. 1A) in untreated

control group. As previously reported, the development of CCA is tightly associated with chronic inflammation, especially caused by the liver fluke infection through oxidative stress (Yongvanit *et al*., 2012). TCTP can serve as an antioxidant agent that could neutralize ROS generated in mammalian cells resulting in protecting them from hydrogen peroxide-induced cell death (Nagano-Ito *et al*., 2009; Lucibello *et al*., 2011). Altogether, these data suggest that TCTP possibly protects cells from oxidative stresses-induced cell death in Ov-associated CCA. Moreover, the TCTP gene was expressed in inflammatory cells at 14 days, during acute infection (Fig. 1B), suggesting that TCTP not only play a role in tumor cells but also in inflammatory cells. According to what MacDonald's group has proposed, secreted TCTP has the ability to induce histamine release, suggesting that TCTP might act as cytokine to alter immune responses at early stage of carcinogenesis (MacDonald *et al*., 1995; Bheekha-Escura *et al*., 2000; Kim *et al*., 2011).

Metastasis is common in CCA progression (Khan *et al*., 2012). Recently, Bae and colleague reported that TCTP is an EMT inducer (Bae *et al*., 2015). Together with our previous study, the silencing of TCTP significantly inhibits cell migration of the M139 CCA cell line (Phanthaphol *et al*., 2014). TCTP might regulate CCA metastasis via EMT pathway. Thus, this data convince us to investigate the alteration of TCTP and EMT markers in CCA. Immunoblotting showed that TCTP regulated SNAIL, an important transcription factor in EMT and metastatic process. However, E-cad and N-cad was slightly affected. These results suggested that there might be other pathways under regulation of SNAIL which contribute to CCA progression and metastasis, rather than EMT alone. The interaction between TCTP-SNAIL and EMT or other pathways is proposed to clarify for the underlying mechanism.

Our findings suggest that TCTP might play roles in the CCA carcinogenesis. TCTP possibly plays a role as EMT regulator in CCA cells via the interaction with SNAIL which confer to the progression of CCA.

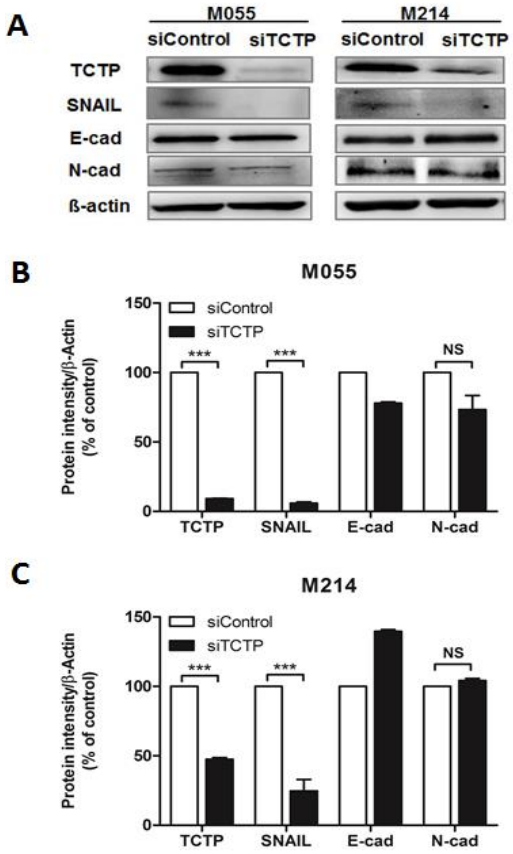

Figure 2 (A) transcription repressors (SNAIL) and its downstream, epithelial marker (E-cadherin) and mesenchymal marker (N-cadherin) were examined by immunoblotting of siControl and siTCTP cells. Graphs present the intensity of protein bands in M055 (B) and M214 (C) that were standardized with ß-actin and scored using Image J software. Values are mean ± SE (***P*< 0.01, ****P*< 0.001, NS; non-significant).

References

Bae SY, Kim HJ, Lee K J, Lee K. Translationally controlled tumor protein induces epithelial to mesenchymal transition and promotes cell migration, invasion and metastasis. *Sci Rep* 2015;doi: 10.1038/srep08061

Bheekha-Escura R, MacGlashan DW, Langdon JM, MacDonald SM. Human recombinant histamine-releasing factor activates human eosinophils and the eosinophilic cell line, AML14-3D10. *Blood* 2000; *96*(6), 2191-8.

Khan SA, Emadossadaty S, Ladep NG, Thomas HC, Elliott P, Taylor-Robinson SD, Toledano MB. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 2012; *56*(4), 848-54.

Kim M, Chung J, Lee C, Jung J, Kwon Y, Lee KA peptide binding to dimerized translationally controlled tumor protein modulates allergic reactions. *J Mol Med (Berl)* 2011; *89*(6), 603-10.

Loilome W, Yongvanit P, Wongkham C, Tepsiri N, Sripa B, Sithithawor P, . . . Miwa M. Altered gene expression in Opisthorchis viverrini-associated cholangiocarcinoma in hamster model. *Mol Carcinog* 2006; *45*(5), 279-87.

Lucibello M, Gambacurta A, Zonfrillo M, Pierimarchi P, Serafino A, Rasi G, . . . Garaci E. TCTP is a critical survival factor that protects cancer cells from oxidative stress-induced cell-death. *Exp Cell Res* 2011; *317*(17), 2479-89.

MacDonald SM, Rafnar T, Langdon J, Lichtenstein LM. Molecular identification of an IgE-dependent histamine-releasing factor. *Science* 1995; *269*(5224), 688-90.

Nagano-Ito M, Banba A, Ichikawa S. Functional cloning of genes that suppress oxidative stress-induced cell death: TCTP prevents hydrogen peroxide-induced cell death. *FEBS Lett* 2009; *583*(8), 1363-7.

Phanthaphol **N**, Loilome W, Techasen A, Yongvanit P, Namwat N. Gene Silencing of Translationally Controlled Tumor Protein (TCTP) by siRNA Inhibits Cell Migration of a Human Cholangiocarcinoma Cell Line. *Srinagarind Med J* 2014; 29 (Suppl); 136-7.

Shin HR, Oh JK, Masuyer E, Curado MP, Bouvard V, Fang YY, Hong ST. Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci* 2010; *101*(3), 579-85.

Sripa B, Pairojkul C. Cholangiocarcinoma: lessons from Thailand. *Curr Opin Gastroentero* 2008; *24*(3), 349-56.

Valle JW, Wasan H, Johnson P, Jones E, Dixon L, Swindell R, . . . Bridgewater J. Gemcitabine alone or in combination with cisplatin in patients with advanced or metastatic cholangiocarcinomas or other biliary tract tumours: a multicenter randomised phase II study - The UK ABC-01 Study. *Br J Cancer* 2009; *101*(4), 621-7.

Yongvanit P, Pinlaor S, Bartsch H. Oxidative and nitrative DNA damage: key events in opisthorchiasis-induced carcinogenesis. *Parasitol Int* 2012; *61*(1), 130-5.