



Gene Silencing of Translationally Controlled Tumor Protein (TCTP) by siRNA Inhibits Cell Migration of a Human Cholangiocarcinoma Cell Line

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Background and Objective: The translationally controlled tumor protein (TCTP) overexpression was founded in many cancers indicating its role in tumorigenesis. We determined the role of TCTP on cell migration of human cholangiocarcinoma (CCA) cell line by RNA interference approach.

Method: The M139 human CCA cell line with high-endogenous TCTP expression was used in this experiment. The effect of small interference RNA (siRNA) based knocking down of TCTP gene on cell migration was evaluated.

Results: The result showed M139 cells transfection with si_TCTP reduced the expression of TCTP protein. The decreased TCTP protein level was associated with decreased cell migration of M139 cells.

Conclusion: This study is the first observation for elucidating the role of TCTP in CCA metastasis. Gene silencing of TCTP by siRNA inhibits cell migration of M139 CCA cell line suggesting that TCTP might be a new targeting molecule in CCA progression.

Keywords: translationally controlled tumor protein (TCTP), cholangiocarcinoma, migration

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Introduction

Translationally controlled tumor protein (TCTP) is a novel tumor-associated protein which is highly expressed in several cancer cell types including cholangiocarcinoma (CCA). Involvement of TCTP in carcinogenesis and cancer progression is well demonstrated and strategies to inhibit TCTP expression level are attractive¹. The highest incidence of this cancer was found in Asia, especially Northeast Thailand. CCA is bile duct cancer that has highly metastasis, commonly leads to the mortality of cancer patients^{2,3}. Therefore, we aimed to investigate the role of TCTP on cell migration in the M139 human CCA cell line by targeting TCTP mRNA using RNA interference approach.

Materials and Methods

We investigated the gene silencing effect of TCTP by RNAi on cell migration of M139 cells. The 8×10^4 cells were seeded in 6-well plate and then transfected with final concentration of 50 nM siRNA, mixture of four siRNAs specific for human TCTP mRNA (Dharmacon, USA), using lipofectamine reagent (Invitrogen, USA). After 48 h of transfection, the cells were collected and the expression of TCTP protein was analyzed by western blot analysis. Directional cell migration ability was analyzed by the wound-healing assay after 48 h transfection. Images of scratched wound were captured at the beginning and at regular intervals during cell migration to close the wound.

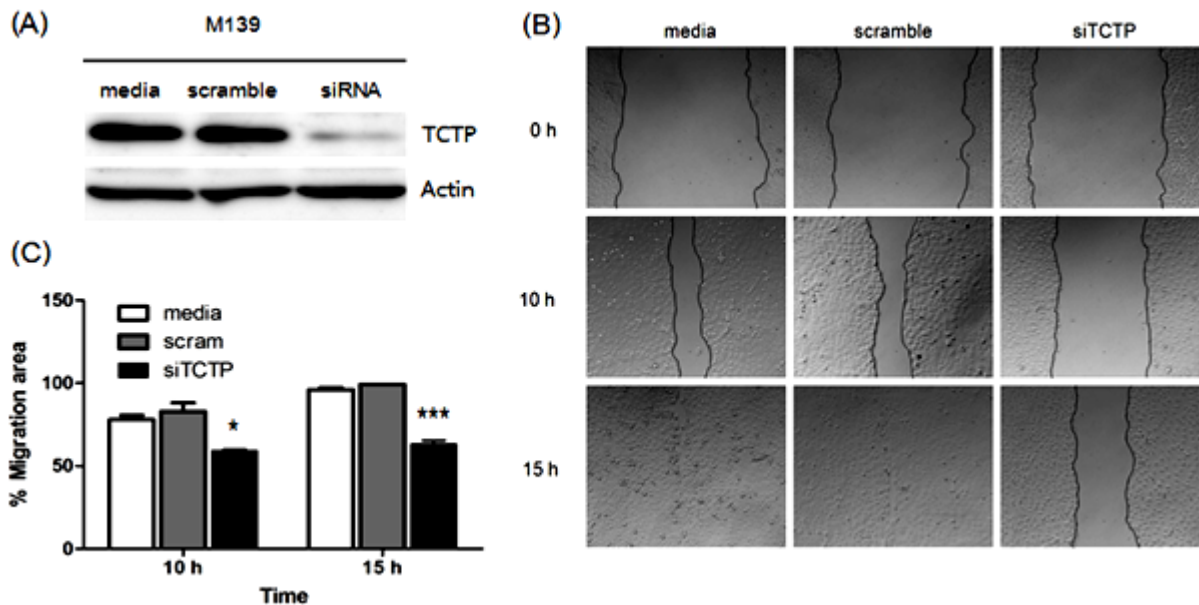


Figure 1 Suppression of TCTP by si_TCTP attenuated the migration activity of M139 cells. (A) After 48 h of transfection, the cells were collected and the expression of TCTP was analyzed by western blot analysis. It was shown that si_TCTP suppressed TCTP protein level in transfected M139 cells. (B) Light microscopy observations show transfected cells with si_TCTP reduced the cell migration of M139 cells compared with non-transfected cells. (C) The Y-axis shows percentage of migration area at 10 h and 15 h during cell migration to close the wound.

Results

After 48 h of transfection, the protein level of TCTP in M139 cells was determined by western blot analysis. Results (fig. 1A) showed that the TCTP protein level was decreased in si_TCTP transfected CCA cells. Following cell migration, cells were visualized under a light microscope. The result (Fig. 1B) showed that the wound of si_TCTP transfected cells was closed significantly slower than in either non-transfected or control siRNA. *p-value less than 0.05 was considered statistically significant compared to the control cells.

Conclusion

TCTP is required for many biological processes. It functions as a pro-survival factor, growth stimulating factor, anti-apoptosis factor and metastasis inducer. In this study, gene silencing of TCTP by RNA interference

significantly inhibits cell migration of the M139 CCA cell line. This study is the first observation for elucidating the role of TCTP in CCA metastasis and TCTP might potentially be a molecular target for CCA treatment.

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