การแสดงออกของยีน *TPT1* ในเซลล์เนื้อเยื่อในโพรงประสาทฟันมนุษย์เมื่อได้รับความร้อน
The Expression of *TPT1* in Heat-treated Human Pulp Cells

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บทคัดย่อ

โปรตีน Translationally controlled tumor protein (TCTP) เป็นโปรตีนที่เกี่ยวข้องกับสภาวะกดดันของเซลล์ โดยจะมีการแสดงออกเพิ่มขึ้นเพื่อปกป้องเซลล์จากสภาวะที่สร้างความเครียดให้แก่เซลล์ ซึ่งงานวิจัยนี้ต้องการศึกษาการแสดงออกของยีน *TPT1* (ยีนที่ผลิตโปรตีน TCTP ในมนุษย์) ในเซลล์เนื้อเยื่อในโพรงประสาทฟันมนุษย์ เมื่ออยู่ในสภาวะกดดันโดยใช้ความร้อน ที่ 43 องศาเซลเซียส เป็นเวลา 45 นาที วิเคราะห์การแสดงออกของยีน *TPT1* ด้วย qPCR จากผลการทดลองพบว่า เซลล์ปกติจะมีการแสดงออกของ *TPT1* ที่ไม่คงที่ และลดลงอย่างต่อเนื่อง ต่ำสุดในชั่วโมงที่ 24 ขณะที่เซลล์ที่ผ่านสภาวะกดดัน มีการแสดงออกอย่างมากสูงสุดในชั่วโมงที่ 24 หลังได้รับความร้อน แสดงให้เห็นว่า โปรตีน TCTP มีการตอบสนองต่อสภาวะกดดันด้วยความร้อน โดยมีการแสดงออกที่แตกต่างจากเซลล์ปกติที่เวลาต่างๆ กัน

ABSTRACT

Translationally controlled tumor protein (TCTP) is usually described as a stress-related protein because of its highly regulated expression in stress conditions for protection against diverse cell stresses. In this study we investigated the expression of *TPT1* (human TCTP's gene) in heat-treated human dental pulp cells (HDPCs). HDPCs were exposed to heat stress at 43 ºC for 45 min, and *TPT1* expression was determined at various time points by quantitative real time polymerase chain reaction (qPCR). Our results show that expression of *TPT1* in heat-treated human pulp cell is up regulated at 24 h after heat stress. However, the level of *TPT1* expression in non-treated cell was not stable and gradually decreased over the time course of the study. These findings indicate that heat stress can modulate *TPT1* expression in HDPCs.

คำสำคัญ: โปรตีน TCTP, ยีน *TPT1*, เซลล์เนื้อเยื่อในโพรงประสาทฟันมนุษย์
Keywords: Translationally controlled tumor protein, *TPT1*, Human dental pulp cells

INTRODUCTION

The translationally controlled tumour protein (TCTP) was discovered about 30 years ago. It is a highly conserved protein and abundantly expressed in all eukaryotes. This protein has been reported in various cellular functions and molecular interactions. TCTP is related to growth promoting, acts as heat shock protein (HSPs) (Gnanasekar *et al*., 2009) and anti-apoptotic properties. Its expression levels are up regulated in response to various cellular stimuli and stresses (Bommer *et al*., 2012). During restorative procedures in carious teeth, cavity preparation can produce heat that induce death signals and lead to apoptosis (Kitamura *et al*., 2005). The intrapulpal temperature rise of 5.5°C can be cause of damage to dental pulp and induced to apoptosis (Lin *et al*., 2010). However, pulp cells may survive such injuries. This may be due to the increased synthesis of HSPs (Amano *et al*., 2006). Several recent studies showed that TCTP plays an important role in cell cycle progression, malignant transformation, early development, and protection against diverse cell stresses such as starvation, heavy metals, calcium or proapoptotic/cytotoxic signals. Less attention has been paid to the effect of heat stress on TCTP expression. Therefore, this study aimed to investigate the expression of *TPT1* in heat-treated human pulp cells.

MATERIALS AND METHODS

1. Primary culture of HDPCs

Normal human third molar was collected from adult (23 years old) at the Dental Hospital, Faculty of Dentistry, Prince of Songkla University, with consent form approved by the Research Ethics Committee, Faculty of Dentistry, Prince of Songkla University. The culture media and supplements were purchased from Invitrogen Corporation, NY, USA unless indicated elsewhere. HDPCs were isolated from freshly extracted sound third molar. Tooth surface was cleaned and cut using a sterile fissure bur to reveal the pulp chamber.

The pulp tissue was minced into pieces and digested in a solution of 3 mg/ml of collagenase Type I in combination with 4 mg/ml of dispase for 1 h at 37°C. After centrifugation, cells were cultured in alpha modified Eagle's medium, αMEM, containing 10% FCS, 100 μM L-ascorbic acid 2-phosphate (Sigma-Aldrich, St Louis, MO, USA), 100 μM L-glutamate, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified incubator containing 5% CO_2. Passage 3 to 5 cells were used in the experiments.

Experimental Design

HDPCs were seeded at 150,000cells/ 35 mm^2 dish. After 24 h, HDPCs were exposed to heat stress, culture medium was changed to medium preheated at 43°C, and culture dishes were placed on blocks preheated to 43°C followed by incubation for 45 minutes at 43°C. After heat stress, medium was changed to medium preheated to 37°C followed by incubation at 37°C (time point 0). Samples were harvested at the specific time points (0, 5 min, 15 min, 1 h, 6 h, and 24 h post-heating). As controls, non–heat-treated HDPCs were cultured at 37°C and harvested at the specific time points (0, 15 min, 6 h, 24 h).

2. Quantitative real time polymerase chain reaction (qPCR)

Total RNA from cell cultures of HDPSs was extracted with RNeasy Mini kit (Qiagen, Crawley, UK). RNA was used to synthesize complementary DNA by SuperScript III Reverse transcriptase (Invitrogen Corporation, NY, USA). Relative mRNA levels were evaluated by qPCR carried out by using the SYBR green (Roch, Mannheim, Germany). Specific primers designed for *TPT1* and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are listed in Table 1. Reactions were carried out at 95°C for 10 minutes followed by 40 cycles of 95°C for 30 seconds, 56°C for 1 minute and 72°C for 30 minute. For data analysis, Relative values were analyzed using ΔΔCT method.

Table 1: Real-time PCR primers (Pilbrow *et al.*, 2008)

Primer		Sequence	PCR Product Length (bp)
GAPDH	Forward	GCTCATTTCCTGGTATGACAACG	213
	Reverse	AGGGGTCTACATGGCAACTG	
TPT1	Forward	AAATGTTAACAAATGTGGCAATTAT	164
	Reverse	AACAATGCCTCCACTCCAAA	

RESULTS AND DISCUSSION

The expression of *TPT1* was confirmed in HDPCs by qPCR analysis. The results (Figure1) showed higher level of *TPT1* expression in heat-stressed HDPCs compared with non–heat-treated. Expression of *TPT1* in control and heat-treated groups was unstable and control group is gradually decreased at 6 and 24 h but *TPT1* was highest up-regulation at 24 h post-heating. This data suggest that *TPT1* is likely to be late-response gene in response to heat stress in HDPCs.

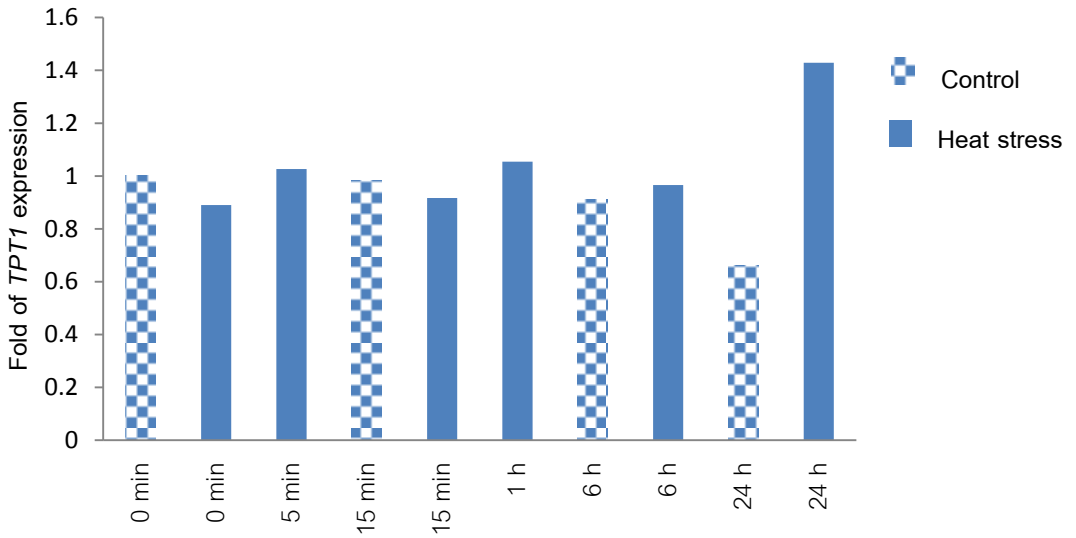


Figure1 qPCR analysis for expression of *TPT1* in non–heat-treated or heat-stressed HDPCs

CONCLUSION

The results of this study showed that heat stress could affect the expression of *TPT1* in pulp cells at various time after the stress exposure.

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