The Expression of TPT1 in Heat-treated Human Pulp Cells

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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 °C 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.

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% CO₂. Passage 3 to 5 cells were used in the experiments.

Experimental Design

HDPCs were seeded at 150,000cells/ 35 mm² 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 ∆∆CT method.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>PCR Product Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>GCTCATTTCTGGTATGACAACG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGGGGTCTACATGGCAACTG</td>
</tr>
<tr>
<td>TPT1</td>
<td>Forward</td>
<td>AAATGTTAACAAATGTGGCAATT</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AACAATGCCTCCACTCCAAA</td>
</tr>
</tbody>
</table>

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](image_url)  
*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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REFERENCES
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