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Expression of translationally controlled tumor protein in heat-stressed human dental pulp cells

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ABSTRACT

Objective: The aim of this study was to investigate the effects of heat stress on cell viability, translationally controlled tumor protein (TCTP) expression, and the effects of recombinant TCTP on heat-stressed human dental pulp cells (HDPCs).
Methods: HDPCs were isolated from human teeth and cultured at 37 °C. For heat stress, HPDCs were incubated at 43 °C for 45 min. After heat stress, recombinant TCTP were added to HDPCs and cultured for various periods of time at 37 °C. Heat-treated cells were then analyzed by DNA staining with Hoechst 33258, MTT, and caspase 3 activity assays. TCTP expression level was assessed by real-time PCR and western blot analysis.
Results: Heat-treated cells displayed lower cell density and nuclear morphology resembling apoptotic body. Heat stress significantly decreased cell viability and induced activity of caspase 3. The effect of recombinant TCTP on pulp cell death from heat stress varied depending on each subject and TCTP concentration. Heat stress up-regulated TCTP mRNA expression level. In contrast, TCTP protein level remained unchanged. Recombinant TCTP did not affect TCTP mRNA expression but down-regulated TCTP protein in heat-treated cells.
Conclusions: Heat stress induces caspase 3 activation and up-regulates TCTP mRNA expression in HDPCs. TCTP did not play a key role on pulp cell recovery from heat stress.

1. Introduction

Dental pulp is a soft tissue capable of self-repair in response to various stimuli such as bacterial toxins, thermal injury, and chemicals from restorative materials. During dental procedures, heat is frequently generated from many sources such as cavity preparation (Vanderlei, Borges, Cavalcanti, & Rode, 2008), laser irradiation (Cavalcanti, Lage-Marques, & Rode, 2003), and the use of light-polymerization system (Uhl, Volpel, & Sigusch, 2006). The amount of heat created during cavity preparation depends on several factors including the drill rotation speed, duration of the temperature rise, and use of the coolant (Ozturk, Usumez, Ozturk, & Ozer, 2004). If high temperature is continuously produced, this may create stress condition to the dental pulp. Prolonged exposure to heat stress can cause pulpal response and severe damage. Intrapulpal temperature increase ranging from 2.2 to 5.9 °C was reported during cavity preparation (Lin, Xu, Lu, & Bai, 2010). Previous study showed that intrapulpal temperature rise of 5.5 °C induced considerable damage, leading to pulpal cell death (Lin et al., 2010).

Depending on the type and extent of the stimuli, cells respond to stress by a wide range of mechanisms such as alteration of gene expression, triggering survival pathways, and activation of death signaling pathways (Fulda, Gorman, Hori, & Samali, 2010). Increase in the synthesis of heat-shock proteins (HSPs) is one of the mechanisms of heat-shock response. HSPs function as molecular chaperones that facilitate refolding of denatured proteins, thus alleviating protein aggregation. Furthermore, HSPs play a role in anti-apoptotic activity, cell survival, and cell recovery from various stress stimuli (Beere, 2005). Previous study showed that HSP25, a rodent homolog of human HSP27, was up-regulated in heat-stressed pulp cells (Lee, Muramatsu, Uekusa, Lee, & Shimono, 2008). Exposure of rat dental pulp cells to heat stress resulted in apoptosis induction (Kitamura et al., 2005). In addition, heat stress was reported to induce interleukin-8 expression, alkaline phosphatase activity, and production of reactive oxygen species (ROS) in human dental pulp cells (HDPCs) (Chang et al., 2009). Limited information is available on the expression of other cellular proteins

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in response to heat stress in human dental pulp cells.

Translationally controlled tumor protein (TCTP) is a highly conserved protein that is ubiquitously expressed in many eukaryotic organisms ranging from yeast, plant, animals. Initially, TCTP was first identified in mouse tumor cells (Bommer & Thiele, 2004) and was highly expressed in many human cancer tissues (Chan, Chen, & Guan, 2012; Lucibello et al., 2011). Further studies revealed that TCTP is a multifunctional protein involved in several cellular processes including cell growth, cell survival, immune response, and stress response (Bommer & Thiele, 2004). Functional study on TCTP-deficient mice demonstrated early embryonic lethality, suggesting its essential role during development (Chen et al., 2007). Down-regulation of TCTP by siRNA showed reduced cell growth and induction of apoptosis in human prostate cancer cells (Gnanasekar, Thirugnanam, Zheng, Chen, & Ramaswamy, 2009), whereas overexpression of TCTP in HeLa cells inhibited etoposide-induced apoptosis and caspase-3 activity (Jung et al., 2014). Recently, TCTP has been described as a survival factor that inhibits cell death induced by oxidative stress (Lucibello et al., 2011). Furthermore, a study has shown that recombinant TCTP can reduce apoptotic cells in 2-hydroxy-ethyl methacrylate (HEMA)-treated dental pulp cells (Wanachottrakul, Chotigeat, & Kedjarune-Leggat, 2011). Taken together, these findings suggested that TCTP is an anti-apoptotic protein that protects cells from various stimuli.

TCTP is expressed in many normal tissues with a wide range of expression levels and is highly up-regulated in response to various extracellular stimuli (Acunzo, Baylot, So, & Rocchi, 2014). Certain cellular conditions such as starvation, heat shock, calcium stress or cytotoxic signals can modulate TCTP expression resulting in either up- or down-regulation of TCTP (Acunzo et al., 2014). We hypothesized that heat stress can modulate TCTP expression and TCTP has a protective role on heat-stressed HDPCs. The aim of this study was to investigate the effects of heat stress on cell viability, TCTP expression, and the effects of recombinant TCTP on heat-stressed HDPCs.

2. Materials and methods

2.1. Protein expression and purification

Banana prawn (*Penaenus merguiensis*) TCTP cDNA was a kind gift from Dr. Wilaiwan Chotigeat (Prince of Songkla University). TCTP cDNA was cloned into the pGEX-4T-1 vector (Amersham, Thailand) as previously described and the resulting construct was called *Pmer*-TCTP (Loongyai, Phongdara, & Chotigeat, 2007). *Escherichia coli* strain BL1 harboring *Pmer*-TCTP was used for recombinant protein expression. An overnight culture of BL1 cells in LB medium containing 100 μg/ml ampicillin was diluted 10-fold with 2 x YT medium containing 100 μg/ml ampicillin. Cells were grown at 37 °C until the cell density at OD_{600} reached 0.5 and were induced with 1 mM IPTG for 3 h. After induction, the cells were harvested by centrifugation and sonicated in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Tris–HCl, pH 8.0). The supernatant was then collected by centrifugation at 10,000 x g for 20 min. The fusion protein was purified using Glutathione Sepharose 4 Fast Flow (GE Healthcare Bio-Science, Piscataway, NJ, USA) following the manufacturer's instructions. *Pmer*-TCTP was released from the GST moiety by thrombin cleavage. The protein concentration was determined by a BCA protein assay kit (Pierce Biotechnology Inc., Rockford, IL, USA) and analyzed on 12% SDS-PAGE.

2.2. Primary cell culture and heat stress

Non-carious human teeth were collected from three human subjects (aged 18–35 years) at the dental hospital, Faculty of Dentistry, Prince of Songkla University. All subjects completed the informed consent approved by the Research Ethics Committee (code no. EC5505-15L). Primary culture of HDPCs was performed using enzymatic method as previously described (Wanachottrakul et al., 2011). Briefly, the tooth was cracked open longitudinally and the pulp tissue was removed and minced into small pieces. Minced pulp tissues were then digested in a solution of 3 mg/ml of collagenase type I (Gibco, Invitrogen, Carlsbad, CA) and 4 mg/ml of dispase (Gibco, Invitrogen, Carlsbad, CA) for 45 min at 37 °C. The dissociated cells were pelleted and cultured in alpha modified Eagle's medium (α-MEM) supplemented with 10% (v/v) fetal calf serum (FCS), 100 μM L-ascorbic acid 2-phosphate (Sigma-Aldrich, St Louis, MO, USA), 100 μM L-glutamate, 100 μg/ml streptomycin, 100 U/ml penicillin, 2.5 μg/ml fungizone. HDPCs were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The cells of passages 3-5 were used for experiments.

For heat stress, HDPCs were exposed to heat stress at 43 °C for 45 min and then incubated at 37 °C (Morotomi et al., 2011). After heat stress, the cells were incubated with various concentrations of *Pmer*-TCTP and then harvested at given time points (0, 5 min, 15 min, 1 h, 6 h, 24 h, 48 h, 72 h) for further analyses. Non-heat-treated cells were used as controls.

2.3. DNA staining

HDPCs were seeded at a density of 1.5×10^5 cells on cover slip in 35-mm dish and incubated for 24 h. Cells were then exposed to heat stress and were stained at given time points. After heat stress, cells were fixed with 3.7% paraformaldehyde for 15 min at room temperature (RT) and permeabilized with 100% methanol for 20 min at RT. Cells were incubated with Hoechst 33258 (0.05 μg/ml) for 15 min at RT. Images were captured using the fluorescent microscope.

2.4. Cell viability assay

Cell viability of HDPCs was examined using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. HDPCs were seeded at a density of 4×10^3 cells/well in 96-well plate and incubated overnight. Subsequently, cells were exposed to heat stress and then treated with various concentrations of *Pmer*-TCTP for 24 and 72 h. After heat stress, 50 μl MTT (Sigma–Aldrich, St. Louis, MO, USA) (5 mg/ml in PBS) was added to each well and incubated in the dark for 4 h at 37 °C. After incubation, the medium was removed and a mixture of 200 μl dimethyl sulfoxide (DMSO) and 25 μl of Sorensen's glycine buffer (0.1 M glycine plus 0.1 M NaCl equilibrated to pH 10.5 with 0.1 M NaOH) was added to dissolve formazan crystals. The absorbance of formazan product was measured at 570 nm. The mean values for each group were obtained from 6 wells. Non-heated groups were set as control. The experiment was repeated 3 times.

2.5. Caspase 3 assay

Caspase 3 activity assay was performed using caspase 3 assay kit (Sigma–Aldrich, St. Louis, MO, USA) following the manufacturer's instruction. The assay is based on detection of *p*-nitroanilide (pNA), which results from the hydrolysis of the substrate acetyl-Asp-Glu-Val-Asp *p*-nitroanilide (Ac-DEVD-pNA) by activated caspase 3. Briefly, cells were lysed in lysis buffer and centrifuged at 18,000 × *g* for 15 min at 4 °C. The cell lysate was then incubated with the caspase substrate in 96-well plate at 37 °C overnight. Absorbance was measured at a wavelength of 405 nm. Caspase activities were expressed as OD value.