Examining the Effect of Oxidative Stress on *Tetrahymena Thermophila* Sirtuin, THD17

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Background Information

Sirtuins are a family of proteins involved in metabolic and chromatin regulation throughout evolution. These proteins modify cellular metabolism in response to altered nutrition or stress (Vassilopoulos, 2011). Sirtuins promote expressions of genes involved in increasing longevity by inhibiting metabolic activity, reducing apoptosis, decreasing the effects of free radicals, and activating DNA repair enzymes (Kumar, 2015). Each sirtuin has specific biological functions and are found in different compartments of the cell.

Tetrahymena thermophila is a unicellular eukaryote which is larger than most mammal cells. *T. thermophila* and humans share more genes with one another than are shared between humans and their more closely related microbial eukaryote model organism, the yeast *S. cerevisiae* (Orias, 2011). The similarity between *T. thermophila* and human cells make them one of the best samples to use when conducting research pertaining to humans. Although numerous studies have been done examining the role of sirtuins in mammals and other organisms, little has been done with *T. thermophila*. *T. thermophila* has about eleven different sirtuins (Slade et al, 2011). Sirtuin THD 17 is the specific gene of interest for this research. THD 17 is similar to sirtuin 5 which is located in the mitochondria (Smith, 2008). Research suggests that mitochondrial sirtuins such as sirt5 directly control the activity of metabolic enzymes and have crucial roles in the metabolic adaptation to dietary conditions such as calorie restriction and fasting (Nakagawa, 2011). Sirtuins have also been studied regarding their effects on oxidative stress and animal cells. A 2013 study revealed that SIRT5 is a critical mediator of oxidative stress induced cell death in cardiomyocytes (Liu, 2013).

Oxidative stress is an imbalance between the production of free radicals and the ability of the body to neutralize their harmful effects with antioxidants. Oxidative stress has been linked to a number of diseases, but most notably linked to accelerated aging in human beings through accelerated cell death. This research study will examine the effects of oxidative stress on *T. thermophila* sirtuin, THD17.

Hypothesis

The hypothesis is that oxidative stress will affect sirtuin THD17 mRNA expression

Materials and Methods

Cell Growth and Stimulation

- *T. thermophila* cells were incubated for 24 hours prior to addition of H_20_2 (0.2 - 0 4 mM). Cells were then incubated for an additional 48 hrs.

RNA Extraction

- mRNA was extracted from *T. thermophila* using the Trizol method. Cells were washed with 10 mM of Tris buffer and 750 ml of Trizol followed by the addition of 0.2 ml of chloroform to each sample. Samples were then incubated for 15 minutes and centrifuged for 15 minutes to separate the phase necessary for extraction.

DCF Stain

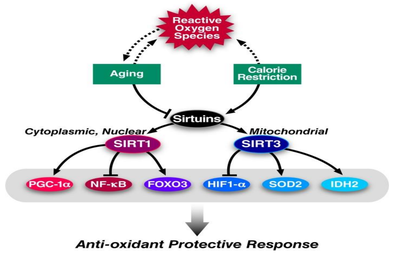
- DCF (2',7'-dichlorofluorescein) was added to 200 µl of cells and incubated for 30 min., following which cells were viewed using a fluorescent microscope.

MTT Assay

- MTT assay was performed to determine the amount of cell survival in each sample of *T. thermophila*. 100 µl of each sample was added to 3 wells. 10 µl of MTT was added to each sample and incubated for 2 hrs. Reagent B was added and read using a plate reader.

CDNA Synthesis

- cDNA synthesis was done using a High-Capacity cDNA Reverse Transcription Kit from ThermoFisher. RNA mix was prepared using the manufacturer's protocol. PCR and Gel Electropheresis were used to analyze samples
- PCR was performed using GoTaq master mix. The primers used were THD17; GAPDH and 18s (control primers); MnSOD, Catalase, GPRX1, GPRX3(Oxidative stress primers). The thermocycler settings for PCR were 95°C for 3 min and 95 C for 30 sec, 56°C for 30 secs, and 72° C for 30 secs for 28 cycles. Samples were analyzed using gel electrophoresis and Alpha imager.



Results

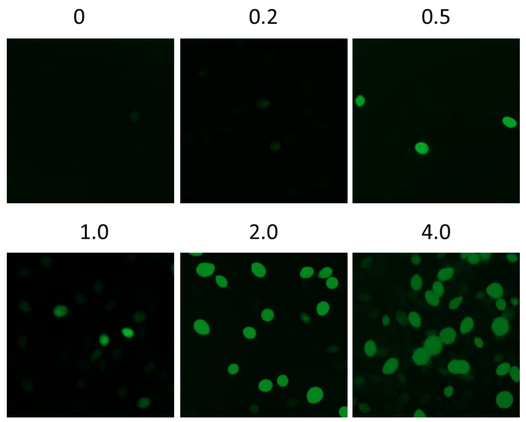
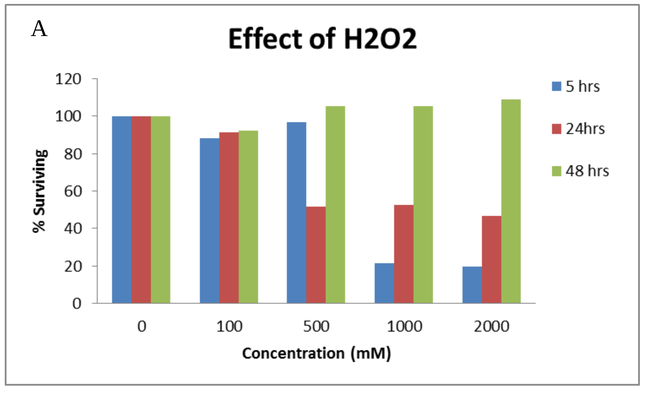


Figure 1. Oxidative Stress in cells stimulated with hydrogen peroxide. Level of oxidative stress is concentration dependent.

MTT Assay



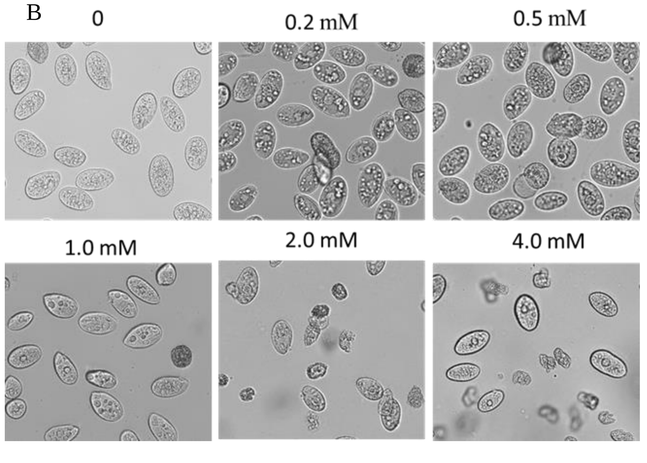


Figure 2. H_2O_2 cell death result. High concentrations of hydrogen peroxide lead to increased cell death. However, cells were able to survive and replicate within 24 hrs.

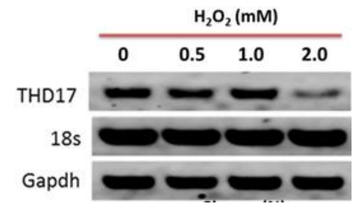


Figure 3. mRNA expression of THD17. 2.0 mM of hydrogen peroxide decreased the expression of THD17.

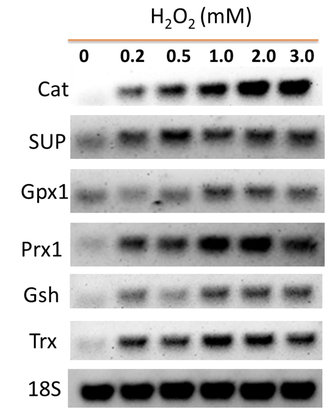


Figure 4. mRNA expression of antioxidant genes. Hydrogen peroxide resulted in increased expression of mRNA levels of antioxidant genes.

Results

- Hydrogen peroxide induced oxidative stress in *T. thermophila* (figure 1). High levels of OS was detected in cells exposed to 1 – 4 mM of hydrogen peroxide.
- Hydrogen peroxide also resulted in increased cell death in a concentration dependent manner (figure 2A). A few cells in 2 and 4 mM treated cells were able to survive and divide in the presence of high levels of oxidative stress.
- The H_2O_2 concentration of 500 µm and 1 mM showed an increase in THD17 mRNA while 2.0 mM lead to reduce expression (figure 3).
- Hydrogen peroxide increased expression levels of antioxidant genes.

Conclusion

- Hydrogen peroxide increased oxidative stress in *T. thermophila* and resulted in high levels of cell death. THD17 and oxidative stress genes also increased in the presence of oxidative stress. These results suggest THD17 may be involved in oxidative stress regulation.

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