Molecular mechanisms of hyperthermia-induced stress response in mammalian cells

Summary of the Ph.D. Thesis

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Introduction

Living beings experience constant changes in their inner and outer environment. For survival, adaption is essential to them. Stress response is an evolutionarily conserved process in which the cell/organism perceives stress, responds to it and copes with it. Its main roles involve maintaining homeostasis, protection against macromolecular damage, and also developing, maintaining and/or enhancing resistance upon stress. The response to stress includes 1) sensing, i.e., the activation of various sensor molecules in response to changed physico-chemical parameters of the environment. This is followed by 2) signaling, in which the perceived stress as signals are engaged in multiple downstream biological cascades to 3) activate compensatory processes. These are repair and cytoprotective processes such as altered expression of genes, increased synthesis or translocation of stress proteins, and also handling damaged macromolecules (like proteins, lipids, RNAs, DNAs) or retailoring membranes. Proper coping means survival and a greater means of stress tolerance, together with the adaption to altered internal and external conditions upon chronic stress.

One of the most studied types of stress is heat. High dose of heat stress (> 42 °C) results in adverse effects to which the cell must respond to, e.g., decreased integrity of the plasma membrane, protein denaturation and aggregation and other means of impaired homeostasis. It is believed, that dealing with harmful conditions, such as elevated temperatures, requires the activation of the heat shock response (HSR). However, pathophysiologically induced mild conditions like fever might also trigger HSR. The "membrane sensor" hypothesis suggests that even a minor change in temperature can modify the fluidity and/or organization of cell membranes, which enables subtle temperature sensing and the activation of the cellular stress response.

Objectives

Studying cellular stress response is key to understand how cells respond to various environmental changes and adapt to new conditions. The main goal of the thesis is to investigate the cellular response, the order of events that occurs as a result of higher temperatures, especially in case of mild, fever-like heat stress in mammalian cells. For this purpose, we were seeking answers to the following questions:

- How do different doses of heat treatments, especially mild fever-like hyperthermia influence cellular growth, protein induction, development of acquired stress tolerance and energy production?
- Is heat shock protein induction - as a main repair mechanism of macromolecular damage - a requirement for the development of acquired stress tolerance?
- What roles can be attributed to modifications of lipid composition/membrane structure upon heat treatments in the development of acquired stress tolerance?
- Which dose dependent signaling pathways are engaged upon mild heat treatments?

Experimental methods

Cell culture

Cell growth (Trypan blue staining and cell count)

Protein measurements (Western Blot)

Viability (resazurin assay)

Survival (colony forming assay)

Cellular energy (luciferase-based ATP measurements)

Mitochondrial membrane potential measurements (JC-1 dye)

Lipidperoxidation assay (DPPP dye)

Lipidomics (electrospray ionization mass spectrometry)

Raft stability analysis by assessing solubility of microdomain resident GFP proteins

Genexpression analysis (RNA sequencing and bioinformatics)

Results

- Upon mild fever-like hyperthermia (40 °C), the rate of cell growth did not change significantly up to 12 hours compared to control (37 °C) in Chinese hamster ovary (CHO) cells, however, 24 hours of heat treatment weakened proliferation.
- Acute (20 min) heat treatments at different temperatures (40 °C, 42.5 °C and 44 °C), followed by a recovery time, led to altered Hsp25 and Hsp70 protein induction in CHO cells. At 40 °C, incubation of 4 h was necessary to detect an increase in the level of Hsp25 without recovery time in CHO cells, while with recovery a shorter heat treatment (40 °C, 2 h) was needed. Heat treatment at 40 °C resulted in no detectable Hsp70 induction up to 12 hours. Such dose of heat stress changed the Hsp25 level in HeLa cells in the same way as in CHO, but the HSP70 induction was different. No change in Hsp25 levels could be detected in MEF cells, and Hsp70 induction can only be observed after 12 hours of heat treatment. Some stress doses were selected for further experiments, short heat stress (40 °C, 1h) led to no induction, while the prolonged one (40 °C, 1h) resulted in a significant increase in the level of Hsp25 in CHO cells independently from recovery. Moderate heat stress (42.5 °C, 1h) produced the highest Hsp25 induction, but only after 6 h of recovery at 37 °C (Peksel et al., 2017; Tiszlavicz et al., 2022).
- The effect of mild fever-like heat stress on survival and viability was studied by using methods based on colony formation and metabolic activity. The challenging stress doses were determined: heat stress (46 °C, 20 min), membrane stress (95 mM benzyl alcohol, 20 min) and oxidative stress (250 µM tert-butyl hydroperoxide, 3 h). Heat treatments at 42.5 and 44 °C for 20 minutes with recovery time led to the development of acquired thermotolerance, which correlates with the induction of Hsps. However, a 20-minute heat treatment (with recovery) at 40 °C resulted in cellular protection even in the absence of Hsp induction. The development of acquired thermotolerance seen as a change in survival and viability due to priming of cells correlated with Hsp induction, however, there was no such straightforward correlation in case of cross-tolerance to benzyl alcohol and tert-butyl hydroperoxide. This suggests that Hsp induction is not a prerequisite for preventing membrane fluidization and oxidative stress (Peksel et al., 2017; Tiszlavicz et al., 2022).

- We detected no significant change in the concentration of ATP as a result of HS at 40 °C and 42.5 °C for 1 hour, however, a slight but significant decrease is observed after 6 hours at 40 °C (Tiszlavicz et al., 2022).
- Mitochondrial membrane potential increases due to mild (40 °C, 1 h) and moderate (42.5 °C, 1 h) heat stress, nonetheless it decreases significantly in the presence of 95 mM benzyl alcohol. Based on our data, only moderate heat treatment prevented depolarization significantly (Tiszlavicz et al., 2022).
- Lipid peroxidation caused by 95 mM benzyl alcohol was attenuated by both mild (40 °C, 1 and 6 hours) and moderate (42.5 °C, 1 hour) heat treatments (Tiszlavicz et al., 2022).
- Heat stress caused well distinguishable dose-dependent changes in the lipidome of CHO cells. Compared to control, more than 200 lipid molecules received significant quantitative change. Longer mild hyperthermia (40 °C, 6 h) resulted in the most significant changes, e.g. an increase in the relative concentration of disaturated and monounsaturated membrane lipid species and lysoglycerophospholipid species together with a decrease in polyunsaturated components. The moderate heat treatment (42.5 °C, 1 hour) resulted in many similar changes to the prolonged mild heat stress, however, the total loss of components containing polyunsaturated fatty acids can only be observed in species containing docosahexaenoic acid. For moderate heat stress, an increase in major sphingolipid species with palmitic acid content was characteristic. The shorter, mild hyperthermia (40 °C, 1 h) caused subtle alterations, which were mostly observed together with the other treatments, e.g. the accumulation of long carbon chain ceramide species with 24:1 fatty acid content, Cer(42:2:2), under all conditions (Tiszlavicz et al., 2022).
- Mild heat treatments at 40 °C are capable to increase the stability of lipid rafts against the release of raft resident fluorescent proteins by detergents and membrane fluidizing agents in CHO cells constitutively expressing the GPI-mGFP protein. Moderate heat stress (42.5 °C, 1 h) and benzyl alcohol have microdomain destabilizing properties.
- As a consequence of heat stress, well distinguishable dose-dependent changes can be observed in the RNAseq transcriptome profiles of CHO cells. Compared to control, the expression of more than 900 genes changed significantly. The genes of the most

enriched pathways by mild heat treatments (40 °C, 1 and 6 h) consist several Hsp and other heat shock response related proteins. Moderate heat stress (42.5 °C, 1 h) resulted rather in the enrichment of signaling pathways associated with inflammation, together with several pathways which includes significantly induced Hsp genes. Based on pathway activation prediction analysis, one of the most significantly enriched and overall activated pathway was the unfolded protein response, especially in case of mild fever-like heat stress (40 °C, 6 hours) (Tiszlavicz et al., 2022).

Summary of results

Mild stress could help cells to survive more severe environmental or pathophysiological conditions. In the course of the doctoral research, we investigated the cellular mechanisms which contribute to the development of stress tolerance upon a prolonged (0–12 h) fever-like (40 °C) or a moderate (42.5 °C) hyperthermia in mammalian CHO cells. Based on our experiments introduced in the thesis:

- In CHO cells, different layers of stress response elicited by different heat dosages highlight the capability of cells to utilize multiple tools to gain tolerance to the same or distinct kind of stress condition.

    - Mild heat triggers a distinct, dose-dependent remodeling of the cellular lipidome followed by the expression of heat shock proteins only at higher heat dosages.
    - A significant elevation in the relative concentration of saturated membrane lipid species and specific lysophosphatidylinositol and sphingolipid species suggests prompt membrane microdomain reorganization and an overall membrane.

- Development of acquired thermotolerance largely depends on Hsp response, however, this is not a prerequisite for building cellular protection.

    - Our results reveal that after a short mild heat stress (40 °C, 20 min), which is followed by recovery, acquired thermotolerance to challenging heat stress (46 °C, 20 min) can be developed even in the absence of induction of heat shock proteins (Hsp25 and Hsp70). Nevertheless, a somewhat longer (40 °C, 1 h) heat treatment did not result in any rise of protective effect, which would otherwise require more time to develop. At the same time, the development of acquired thermotolerance correlated with Hsp induction when cells were pretreated with higher doses of heat stress (40 °C, 6 h or 42.5 °C, 1 h).

- Acquired cross tolerance to membrane and oxidative stress can be developed by mild and moderate heat treatment.

    - Our results show that regardless of the induction of heat shock proteins (Hsp25 and Hsp70), acquired cross tolerance to membrane perturbing agent (benzyl alcohol) and oxidizing agent (TBHP) might be developed upon mild fever-like hyperthermia (40 °C). Therefore, the development of acquired cross tolerance is a result of another cytoprotective mechanism other than Hsp response, such as membrane retailoring or the unfolded protein response (UPR).

- Mild fever-like hyperthermia initiates endoplasmic reticulum stress-related signaling cascades resulting in lipid rearrangement and ultimately in an elevated resistance against membrane fluidization by benzyl alcohol.

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    - RNAseq data of heat-treated CHO cells revealed that one of the most enriched pathways was the UPR, which was previously considered to be activated by the accumulation of misfolded proteins and by lipid bilayer stress as well, particularly to the decrease of membrane fluidity. Here, we showed that the increase of membrane fluidity could also trigger the activation of the UPR, which is supported by prediction analyses, especially in the case of long mild heat stress (40 °C, 6 h). This finding was further underpinned by lipidomic data: the rise of relative concentration of lisolipids due to hyperthermia generates lipid bilayer stress. In addition, the association of lysophosphatidylinositol and GPR55 comprises a considerable role in endoplasmic reticulum stress response.

Összefoglaló

Az élőlények képesek érzékelni a belső és külső környezeti változásokat, és azokra többféle módon reagálnak. A doktori munka során megvizsgáltuk, hogy a stresszre válaszolva, különösen enyhe lázszerű hipertermia esetén milyen sejtszintű mechanizmusok lépnek működésbe emlőssejtekben. Tanulmányoztuk, hogy az enyhe (40 °C) hosszan tartó (0-12 óra) és a mérsékelt (42,5 °C, 1 óra) hőstressz hogyan befolyásolja a sejtnövekedést, a Hsp indukciót, a szerzett stressztolerancia kialakulását, sejt energiaállapotát, lipidperoxidáció mértékét, lipidomikát, raft stabilitást és a génexpressziót kínai hörcsög petefészek (CHO) sejtekben. Azt találtuk, hogy az enyhe hőstressz a lipidprofil dózisfüggő átalakulását eredményezi, amit a magasabb dózisok esetén a hősokkfehérjék expresszió követ. A telített membrán lipidspecieszek és a specifikus lizofoszfatidil-inozit és szfingolipid specieszek relatív koncentrációjának szignifikáns emelkedése arra utal, hogy a membrán mikrodomének gyorsan átszerveződnek és a membránok rigiditása növekszik időfüggő módon, ami a sejt válasza hő membrán fluidizáló hatásával szemben. Az RNAseq kísérletek alapján az enyhe hő aktiválja az endoplazmatikus retikulum stresszhez kapcsolódó jelátviteli kaszkádokat, amely lipid-átrendeződéshez és végső soron benzil-alkohol okozta membránfluidizációval szembeni megnövekedett rezisztenciához vezet. Az akár letális, fehérje denaturációra képes magas hőmérsékletek esetén a védelemhez a klasszikus hősokk fehérjeválaszra van szükség. A különböző hődózisok különböző stresszválaszt váltanak ki, ami rávilágít arra, hogy a sejtek képesek többféle eszközt is felhasználni a stresszel szembeni ellenállás illetve a túlélés érdekében.

Publications

MTMT identification number: 10062140

Publications related to the thesis:

1. Peksel, B., Gombos, I., Péter, M., Vigh, L., **Tiszlavicz, Á.**, Brameshuber, M., Balogh, G., Schütz, G. J., Horváth, I., Vigh, L., & Török, Z. (2017). Mild heat induces a distinct "eustress" response in Chinese Hamster Ovary cells but does not induce heat shock protein synthesis. Scientific Reports, 7(1), 15643. https://doi.org/10.1038/s41598-017-15821-8

   IF: **4.122** (2017)

2. **Tiszlavicz, Á.**, Gombos, I., Péter, M., Hegedűs, Z., Hunya, Á., Dukic, B., Nagy, I., Peksel, B., Balogh, G., Horváth, I., Vígh, L., & Török, Z. (2022). Distinct Cellular Tools of Mild Hyperthermia-Induced Acquired Stress Tolerance in Chinese Hamster Ovary Cells. Biomedicines, 10(5), 24. https://doi.org/10.3390/biomedicines10051172

   IF: **4.757** (2021)

Other publications:

3. Farsang, A., Szatmári, J., Bartus, M., **Tiszlavicz, Á.**, & Barta, K. (2022). Quantification of deflation-induced soil loss on chernozems: Field protocol and sediment trap development based on wind tunnel experiments. Zeitschrift Für Geomorphologie, 63(4), 329–341. https://doi.org/10.1127/zfg/2021/0709

   IF: **1.571** (2021)

Cumulative IF of journals: **10.45**

Cumulative IF of journals (related to the thesis): **8.879**

Conference participations:

- Dukic B., **Tiszlavicz Á**., Gombos I., Péter M., Balogh G., Varga-Zsíros V., Hunya Á., Vígh L., Török Zs: Investigation of membrane-coupled events induced by fever-like heat treatment, 2022. Május 25-27., Straub-Napok, Szeged, poszter
- Dukic B., **Tiszlavicz Á**., Gombos I., Peksel B., Péter M., Balogh G., Varga-Zsíros V., Hunya Á., Horváth I., Vígh L., Török Zs: Lázszerű hőkezeléssel indukált membránkapcsolt események vizsgálata, 2021. november 16-19., 50. Membrán Transzport Konferencia, Sümeg, poszter
- **Tiszlavicz Á**., Dukic B., Gombos I., Peksel B., Péter M., Balogh G., Horváth I., Vigh L., Török Zs.: Enyhe hipertermia-okozta szerzett stresszrezisztencia molekuláris mechanizmusa emlős sejtekben, 2021. november 16-19., 50. Membrán Transzport Konferencia, Sümeg, poszter
- Dukic B., **Tiszlavicz Á**., Gombos I., Peksel B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Interdiszciplináris Doktorandusz Konferencia, 2020. november 28., online előadás
- Dukic B., **Tiszlavicz Á**., Gombos I., Peksel B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Lázszerű hőkezeléssel indukált membránkapcsolt jelátviteli események vizsgálata, XIII. Tavaszi Szél Konferencia, 2020. október 16., online előadás
- **Tiszlavicz Á**., Gombos I., Peksel B., Dukic B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Acquired cellular stress resistance in the absence of heat shock protein induction, XXII. Annual Linz Winter Workshop, 2020. január 31-február 3., Linz (AU), poszter
- Dukic B., **Tiszlavicz Á**., Gombos I., Peksel B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Mild heat stress-induced early cellular events in mammalian cells, EMBO EMBL - Seeing is believing, 2019. október 9-12., Heidelberg, poszter
- Dukic B., **Tiszlavicz Á**., Gombos I., Peksel B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Mild heat stress-induced early cellular events in mammalian cells, Straub-Napok, 2019. május 30-31., Szeged, poszter
- **Tiszlavicz Á**., Gombos I., Peksel B., Dukic B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs: Acquired cellular stress resistance in the absence of heat shock protein induction, Straub-Napok, 2019. május 30-31., Szeged, poszter
- Dukic B., **Tiszlavicz Á**., Gombos I., Peksel B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Enyhe hőstressz okozta korai események vizsgálata emlős sejtekben, 49. Membrán Transzport Konferencia, 2019. május 14-17., Sümeg, poszter
- **Tiszlavicz Á**., Gombos I., Peksel B., Dukic B., Péter M., Balogh G., Horváth I., Vígh L., Török Zs.: Acquired cellular stress resistance in the absence of heat shock protein induction, Hungarian Molecular Life Sciences, 2019. március 29-31., Eger, poszter