



Summary of the PhD thesis



# **Regulatory role of plasma membrane microdomains in cellular stress sensing and growth signaling**

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## Introduction

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By being the outermost boundary of the cell, the plasma membrane is deeply involved in the sensing and transmission of signals from the environment towards the cellular interior. Lipid rafts are creating a great degree of membrane heterogeneity and by this they are allowing a higher level of compartmentalization to happen in the plasma membrane. This heterogeneity has a great significance in signaling processes at the membrane level, as the compartmentalized nature allows the lipid rafts to act like sorting platforms for signal transduction proteins. Many proteins assembled in lipid rafts are involved in a wide range of signaling processes ranging from stress sensing, cell survival, growth signaling to adhesion and migration. An important defining nature of these platforms is that they are highly dynamic and capable of rapid changes. It means that these sorting structures of the membrane can react to rapid changes in the environment in a very short time and can re-establish their basal state after the loss of the signal in the same rapid way thus allowing a precise regulation over cellular signaling and a prone and timely response to environmental signals. Our work concentrates on two aspects of the membrane raft regulated cellular signaling.

The first part of the thesis is concentrating on aspects of the phenomena of the membrane sensor model aiming to understand the reaction of a cell to membrane structural changes by introducing artificial membrane fluidizing agents and analyzing the expression and function of the stress proteins induced by the procedure. In the long term these set of experiments are looking for the answer to the question: how can a cell maintain its membrane integrity under challenging conditions?

The second part of the thesis is examining a clinically relevant protein associated with cancer progression. The underlying work is wishing to analyze the mechanism of action of this oncogene in the context of its membrane association with a further aim to delineate and define its precise signaling mechanism which could be relevant in describing its function as a cancer promoting factor and for designing future therapies against it.

## Aims of the Thesis

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The work detailed in this thesis is concentrating on revealing cellular phenomenas within the context of their plasma membrane association. The main goal of the thesis was to characterize physiologically and pathologically relevant events at the level of the plasma membrane and couple these to the regulatory role of detergent resistant plasma membrane microdomains. In order to gain a deeper understanding on how the plasma membrane works in times of stress and disease. The questions addressed are as follows.

- **On the level of membrane regulated stress signaling and adaptation:**
  - What is the extent of protein expression of the small heat shock protein HSP25 upon artificial membrane fluidization in mammalian cells?
  - Does the presence of this HSP affects the survival of cells under stress conditions?
  - Is the HSP25 associates with the plasma membrane or affects the structure of the membrane in order to maintain membrane homeostasis?
  
- **On the level of membrane associated oncogenesis:**
  - In which extent does the oncogenic PRL3 protein is being expressed in melanoma cells and if there is any factor associated with its expression?
  - Does the expression of the PRL3 protein affects the tumorigenic properties of melanoma cells?
  - Does the PRL3 protein associates with any specific regions of the plasma membrane?
  - Is there a sub-membrane specific proteome that is affected by the PRL3 which can lead to oncogenesis?

## Materials and methods

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Cell culture and treatments

Transfection, clone selection and stable expression

Western blot analysis

Immunoprecipitation

Rac1-GTP pull-down assay

Soft agar assay

Tumor growth assay

Plasma membrane isolation

Detergent-free purification of caveolin enriched membrane fractions

Confocal microscopy

Imaging of di-4-ANNEPDHQ and calculation of General Polarization (GP)

Preparation of plasma membrane sheets

Domain size analysis of sphingomyelin rich microdomains

Electron microscopy

## Summary of findings

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The findings obtained during the current thesis work are summarized as follows:

- **On the level of membrane regulated stress signaling and adaptation:**
  - By applying the artificial membrane fluidizer benzyl alcohol, we observed an elevated expression of HSP25 at the first time at the protein level.
  - Our investigations has led to the discovery of a so far unknown feature of HSP25, which was materializing in its effect to balance the effect of forced membrane fluidization by acting as a membrane buffering agent.
  - The effect of HSP25 on the membrane was shown to represent itself in the promotion of membrane domain clustering as the smaller domains were converted into larger ones purely upon the expression of HSP25. This effect was further substantiated by our fluorescent correlation spectroscopy measurements where the elevated confinement values were pointing towards a more compartmentalized membrane structure upon HSP25 expression.
  
- **On the level of membrane associated oncogenesis:**
  - Here we identified PRL3 as a protein upregulated in a time and dose dependent manner upon treatment with several clinically relevant anti-tumor therapeutics.
  - Our experiments were able to successfully connect the presence of PRL3 with a distinct phenotype that could further tumor progression by affecting tumor growth.
  - PRL3 was shown to be affect the structure of the membrane and being associated with the caveolae type lipid raft structures of the plasma membrane
  - PRL3 was further associated with the caveolae proteome as it has been detected to dephosphorylate integrin beta 1 at its Thr788/789 site.
  - PRL3 expression was also associated with increased plasma membrane targeting to the caveolae region and subsequent activation of Rac1.
  - Rac1 was affiliated with the activation of the following signaling pathway: Rac1-GTP/PAK1/AKT/GSK3 $\beta$  which lead to the nuclear accumulation of the cyclin D1 molecule.

## **Publications related to the thesis**

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MTMT number: 10038350

### **Chemotherapy induced PRL3 expression promotes cancer growth via plasma membrane remodeling and specific alterations of caveolae-associated signaling**

**Csobozy B**, Gombos I, Tatrai E, Tovari J, Kiss A, Horvath I, Vigh L

Cell Communication and Signaling (under review)

I.F.: 3.91

### **Membrane fluidity matters: hyperthermia from the aspects of lipids and membranes.**

**Csobozy B**, Balogh GE, Kusz E, Gombos I, Peter M, Crul T, Gungor B, Haracska L, Bogdanovics G, Torok Z, Horvath I, Vigh L.

Int J Hyperthermia. 2013 Aug; 29(5):491-9. doi: 10.3109/02656736.2013.808765. Epub 2013 Jul 10.

I.F.: 2.645

### **Plasma membranes as heat stress sensors: from lipid-controlled molecular switches to therapeutic applications.**

Török Z, Crul T, Maresca B, Schütz GJ, Viana F, Dindia L, Piotto S, Brameshuber M, Balogh G, Péter M, Porta A, Trapani A, Gombos I, Glatz A, Gungor B, Peksel B, Vigh L Jr, **Csobozy B**, Horváth I, Vijayan MM, Hooper PL, Harwood JL, Vigh L.

Biochim Biophys Acta. 2014 Jun; 1838(6):1594-618. doi: 10.1016/j.bbamem.2013.12.015. Epub 2013 Dec 27.

I.F.: 3,498

## DECLARATION

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Hereby, as corresponding author and supervisor I declare the role of Balint Csoboz in the following publications;

Balint Csoboz, Gabor E Balogh, Erzsebet Kusz, Imre Gombos, Maria Peter, Tim Crul, Burcin Gungor, Lajos Haracska, Gordana Bogdanovics, Zsolt Torok, Ibolya Horvath, Laszlo Vigh.

Membrane fluidity matters: hyperthermia from the aspects of lipids and membranes. (2013) Int J Hyperthermia. 2013 Aug; 29(5):491-9. doi: 10.3109/02656736.2013.808765. Epub 2013 Jul 10.

All the experiments were carried out by Balint Csoboz. He was also involved in the writing and editing of the manuscript. I also declare that those parts in the above mentioned articles were not or will not be used in the past or in the future, respectively for the purpose of acquiring an academic degree or title.

Prof. Dr. László Víg

Szeged, 17.05.2018

Hereby, as corresponding author I declare the role of Balint Csoboz in the following publication;

Török, Zsolt, Tim Crul, Bruno Maresca, Gerhard J Schütz, Felix Viana, Laura Dindia, Stefano Piotto, Mario Brameshuber, Gabor Balogh, Maria Peter, Amalia Porta, Alfonso Trapani, Imre Gombos, Attila Glatz, Burcin Güngör, Begüm Peksel, Laszlo Jr Vigh, **Balint Csoboz**, Ibolya Horvath, Mathilakath M. Vijayan, Phillip L. Hooper, John L. Harwood, Laszlo Vigh. "Plasma Membranes as Heat Stress Sensors: From Lipid-Controlled Molecular Switches to Therapeutic Applications." Biochimica et Biophysica Acta 1838, no. 6 (June 2014): 1594–1618. doi:10.1016/j.bbamem.2013.12.015.

This is a review article including several authors. Balint Csoboz was actively taken part in collecting literature and writing chapter 4.: Stress hormones and the membrane-regulated stress response: heat shock can alter membranes indirectly through the elevation of plasma glucocorticoid levels. I also declare that those parts in the above mentioned article were not or will not be used in the past or in the future, respectively for the purpose of acquiring an academic degree or title.

Dr. Zsolt TÖRÖK

Szeged, 17.05.2018