Summary of the PhD thesis

The role of small GTPase Rac1 in stress signaling

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INTRODUCTION

All organisms are exposed to many external challenges now and then such as elevated temperatures, oxidative stress and hypoxia etc. resulting in protein denaturation and DNA damage. Not only the environmental factors but also pathophysiological conditions - such as neurodegenerative diseases, diabetes and cancer -can act as stress factors. Most of these insults are also reported to target membrane lipids as well. A necessary condition for survival is to develop an adaptation against a variety of unpredictable changes. Therefore, cells utilize complex, specialized defense systems and induce rapid, temporary response called "stress response" to maintain a stable intracellular milieu. Heat stress induces complex signaling pathways to respond for survival. Based on the severity and duration of stress, heat expositions can be classified as mild heat and severe heat. Mild heat stress is presumed to positively regulate cell cycle progression and differentiation triggering a complex cascade of events including Rac1 signaling pathway.

Heat shock or stress response is first characterized by the expression of heat shock proteins (HSPs) which promote the cellular recovery and thermotolerance. They are highly conserved proteins and can be activated by various types of stress. HSPs are named according to their molecular weights: HSP100, HSP90, HSP70, HSP60 and the "small HSPs". They can reside in different compartments of the cell and can associate with plasma membrane (PM) via their specific protein and lipid interactions. They are molecular chaperons that bind and stabilize proteins with instable conformation and assist in proper folding of misfolded proteins to achieve their native forms. There is a growing body of evidence linking the HSP expression induced by a variety of stress conditions to changes in the lipid composition and in the architecture of membranes. According to the "membrane thermosensor" hypothesis, heat stress can be sensed through subtle changes in the fluidity and micro-domain hyperstructures of membranes influencing membrane-localized stress sensing and signaling and hence the expression of HSPs.

Having more information about the PM structure and function has opened up new doors to understand its role in the complexity of the cellular interactions. Lipid microdomains are the hyperstructures of cholesterol (Chol) and saturated lipid containing liquid ordered patches of PM. Specific signaling proteins are targeted to these microdomains as consequence of their lipidated features. The Rho family small GTPase Rac1 is an important integrator of signals from growth factor receptors, integrins and altered signaling related to cell transformation, tumor invasion, and metastasis. Rac1 is known to promote actin

assembly and have an important role in the formation of lamellipodia and membrane ruffles. Rac1 associates with Chol rich PM microdomains under growth factor stimuli. Rac1 is also known to drive actin polymerization which can be induced by mild hyperthermia. Moreover, Rac1 can effect HSP expression under the mild heat conditions where cells perceive the mild heat as an external stimulation rather than an insult and respond accordingly.

In favor of membrane thermosensor model, our current work hypothesizes that Rac1 pathway is involved in stress signaling through the effect of heat stress on membrane micro-domain organization. Membrane rearrangement and/or membrane hyperfluidization by mild heat stress may activate growth factor receptor tyrosine kinases by causing their non-specific clustering. Activation of such cell surface receptors can stimulate PI3K which in turn activates the Rac1. Activated Rac1 is then released from its Rho-GDI complex which keeps Rac1 solubilized in cytosol by shielding its geranyl-geranyl group and Rac1 undergoes reversible palmitoylation, then subsequent translocation to the Chol rich domain of PM. After, membrane localized Rac1 interacts with its effector, p21 activated kinase (Pak1), which also mediates a downstream signaling cascade to MAPKs such as p38MAPK, affecting HSP expression.

AIMS OF THE STUDY

Our aim was to explore the possible involvement of Rac1 in stress signaling in mammalian cells. The main goals of the study were:

- to test the Rac1 PM membrane localization upon heat stress
- to monitor the PM micro-domain alterations in connection with Rac1 localization and activity.
- to demonstrate the effect of Rac1 on cytoskeletal changes upon heat stress.
- to figure out the involvement of Rac1 in HSP expression and,
- to check whether Rac1 contributes to the pathway(s) of HSP induction by membrane interacting HSP co-inducer drug candidates.

METHODS AND TECHNICS

Cell culturing

Transient transfection

Plasma membrane and crude membrane isolation

Western blotting

Quantitative reverse transcription PCR (qRT-PCR)

Confocal laser scanning microscopy

Scanning electron microscopy (SEM)

Total internal reflection fluorescent microscopy (TIRF)

Flow cytometry

SUMMARY OF FINDINGS

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

1. Rac1 is a membrane binding protein and stress conditions tested clearly induced Rac1 PM association. Functionally inactive Rac1 can attain membrane binding meaning that though activity requires membrane binding, but membrane binding does not go "hand in hand" with activity. Palmitoylation of Rac1 is a prerequisite for its PM localization.
2. Heat stress conditions cause PM micro-domain enlargements. Palmitoylation of Rac1 can be one of the major regulatory steps for the appearance of larger membrane micro-domains, acting as stress signaling platforms, upon heat stress.
3. Severe heat shock conditions are resulted in F-actin filament fragmentation. Rac1 is one of the important downstream elements of cytoskeletal organization and cell shape alterations as its inhibition saves intact F-actin and conserves the cell shape under severe heat stress conditions.
4. Activation of Rac1 influences the inducible *hsp25* and *hsp70* expressions both at mRNA and protein levels under mild and severe heat stress conditions, which also trigger Rac1 localization to the PM. Rac1 also controls the operation of BGP15-mediated HSP activation, via acting as raft-stabilizer.
5. Rac1 regulates the phosphorylation levels of p38MAPK under mild heat conditions. Although data obtained doesn't confirm any change in Rac1-mediated HSF1 hyperphosphorylation but we can assume that active p38MAPK may exhibit inhibiting phosphorylation on HSF1 which in turn may result in decreased HSP

response. Elucidation of the exact mechanism of Rac1-mediated HSP regulation needs therefore further investigations.

PUBLICATIONS RELATED TO THIS THESIS

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