Abstract

Maintaining amino acid homeostasis in the cell requires effective regulatory mechanisms, including signaling pathways involving such kinases as mTOR and GCN2 coordinated with the work of specialized membrane proteins that form a complex and interdependent transport network. The number and activity of amino acid transporters in the plasma membrane is closely coupled with the cell's demand for amino acids. Many amino acids transporters are glycoproteins that undergo the N-glycosylation process in the endoplasmic reticulum and the Golgi apparatus which determines their maturation, localization and transport activity.

The main purpose of this doctoral dissertation was to determine the importance of the N-glycosylation process in the Golgi apparatus in the functioning of the membrane amino acid transporter SNAT2 which is an important element in the adaptation to hyperosmotic stress conditions. In addition, the aim was to increase knowledge about the adaptive mechanisms triggered in the cell in response to increased extracellular osmolarity. The third objective was to investigate the role of osmoadaptive accumulation of amino acids in the regulation of the ERK1/2 kinase signaling pathway induced in response to hyperosmotic stress.

As part of the completed research work it was shown that adaptation to the conditions of increased osmolarity is completely independent of the integrated stress response (ISR) signaling pathway. The unique mechanism of osmoadaptation involves the induction of GADD34 and SNAT2. The mature form of the SNAT2 protein, N-glycosylated in the endoplasmic reticulum and Golgi apparatus localizes to the plasma membrane and enables the uptake of amino acids. It has been shown that the interaction of SNAT2 and its dependent antiporters LAT1 and ASCT1 ensures the restoration of homeostasis in cells and determines the reactivation of mTORC1 which ensures efficient translation - initially inhibited in response to osmotic stress. The data collected suggest that induction of the SNAT2 transporter is a key part of the transcriptional program of the tonicity enhancer binding protein – TonEBP that protects cells from apoptosis.

In the second part of the study, the effect of disorder terminal N-glycosylation in *trans*-Golgi cisterns on the maturation, localization and activity of SNAT2 as well as adaptation to hyperosmotic stress conditions was determined. Deficiency of UDP-galactose in *trans*-Golgi cisterns caused by knock-out gene encoding the SLC35A2 transporter indicates that modifications of the SNAT2 N-glycan structure in this cellular compartment are necessary

for the proper functioning of the glycoprotein. Disturbance of the *trans*-Golgi function prevents the delivery of SNAT2 to the plasma membrane and its transport function, which causes significant perturbations in intracellular amino acid levels, preventing adaptation. These observations were confirmed by analyzes using an inhibitor of tubulin polymerization - nocodazole, leading to fragmentation of the Golgi apparatus. The disruption of organelle integrity had a negative impact on the maturation and maintenance of SNAT2 transport function. This indicates the important role of the Golgi apparatus in the adaptation to hyperosmotic stress and the control of amino acid metabolism.

The last step of the research was to determine the importance of osmoadaptive accumulation of amino acids in the regulation of the ERK1/2 kinase signaling pathway activated during hyperosmotic stress independently of growth factors. A two-phase mechanism was presented assuming that macromolecular crowding in cells exposed to increased extracellular osmolarity and the accompanying decrease in the level of polyamines leads to the liquid-liquid phase separation (LLPS) of the SHP2 phosphatase promoting hyperactivation of the ERK1/2 kinase. Amino acids accumulated via SNAT2 and SNAT2-dependent exchangers disperse the formed biomolecular condensates of SHP2 resulting in kinase inactivation during osmoadaptation. These observations allow to conclude that amine compounds (polyamines and amino acids) play an important role in the formation/dispersion of biomolecular condensates and regulation of ERK1/2 kinase activity.

In conclusion, modifications of the N-glycan of the SNAT2 transporter in the Golgi apparatus are an integral part of adaptation to hyperosmotic stress. The accumulation of amino acids via a complex network of amino acid transporters in cells exposed to stress not only allows for the restoration of homeostasis but also serves as a modulator of ERK1/2 kinase activity. Therefore, a thorough understanding of the functions of the Golgi apparatus opens the way to a better understanding of cell metabolism, and above all, the phenomenon of adaptation to stress conditions.

Key words: Golgi apparatus, N-glycosylation, SNAT2, hyperosmotic stress, ERK1/2

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