No. 0638

Exhaustive Analysis of Signal Transduction Involved in Mobilization of Intracellular NaCl Changed by Hypertonicity

Nobuyuki Takahashi, Takahiro Shimizu, Hana Inoue

Department of Cell Physiology, National Institute of Physiological Sciences

Summary

Almost cells show regulatory volume increase (RVI), cell volume recovery from shrinkage induced under extracellular hypertonic conditions. In RVI, water inflows into cells following to NaCl absorption through ion transporters such as Na^+/H^+ exchangers (NHE). However, under apoptotic conditions, cell volume persistently decreases without RVI. The sustained cell shrinkage is a major hallmark of apoptotic cell death. This suggests that RVI should be inhibited to induce apoptotic cell death. In previous work, it has been indicated that Akt activated by extracellular hypertonicity is indispensable for RVI (NaCl absorption) and that ASK, which is activated by apoptotic stimuli, inhibits Akt activation to suppress RVI under hypertonic conditions. Next questions are “How is Akt activated by hypertonicity to induce NaCl absorption and inhibited by ASK under apoptotic conditions?” and “How is NaCl absorption to induce RVI important for cell survival?”

To detect protein phosphorylation induced by hypertonicity, 2-dementional electrophoresis of hypertonicity-treated and control HeLa cell protein samples were performed. After purification of phospho-proteins, many proteins were newly phosphorylated by hypertonicity. Now identification of these phospho-proteins was tried by using mass spectrometry. On the other hand, when RVI was inhibited by combined application of NHE and anion exchanger blockers, hypertonic stress induced prolonged shrinkage followed by caspase-3 activation in HeLa cells. Hypertonicity also induced apoptosis in NHE1-deficient PS120 fibroblasts, which lack the RVI response. When RVI was restored by transfection of these cells with NHE1, hypertonicity-induced apoptosis was completely prevented. Thus, it is concluded that RVI dysfunction is indispensable for the persistence of AVD and induction of apoptosis.