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Analysis of volume-dependent cellular NaCl absorption and inhibition of the absorption during apoptotic cell death.

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Summary

Almost cells show cell volume recovery from shrinkage induced by extracellular hypertonicity (regulatory volume increase; RVI), in which water molecules inflow into cytosol following to NaCl absorption. However, under apoptotic conditions, cell volume persistently decreases without RVI. This indicates that RVI is inhibited under apoptotic conditions. Therefore, followed experiments were performed to answer to questions, “How is RVI via NaCl absorption induced by hypertonicity ?” and “How is RVI via NaCl absorption inhibited by apoptotic stimuli ?”

RVI via NaCl absorption of human epithelial cells, HeLa cells, was significantly inhibited by Akt inhibitor under hypertonic conditions. Moreover, exogenous expression of Akt dominant negative form also inhibited the RVI of HeLa cells. Akt was phosphorylated by hypertonicity in HeLa cells. This phosphorylation of Akt was inhibited by various apoptotic stimuli such as either application of staurosporine, H_2O_2, or TNF-α, which can induce apoptosis and suppress RVI in HeLa cells. Next, to elucidate molecular mechanism of RVI inhibition by apoptotic stimuli, we examined the involvement of apoptosis signal-regulating kinase 1 (ASK1), which is activated by apoptotic stimuli described above. Overexpression of ASK1 kinase dead mutant restored the Akt phosphorylation and RVI under apoptotic conditions.

Thus, it is possible that Akt is activated by hypertonicity to induces volume-dependent NaCl absorption (RVI) in HeLa cells and that the activation of Akt is inhibited by ASK1 activated by various apoptotic stimuli so that persistent cell shrinkage occurs without the volume-dependent NaCl absorption to cause apoptotic cell death.