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Exhaustive Analysis of Protein Phosphorylation Involved in NaCl Uptake under High Concentration of NaCl

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Summary

Under the conditions of high concentration of NaCl, almost cells show regulatory volume increase (RVI), which is cell volume recovery from shrinkage, through NaCl uptake. Activation of Na⁺/H⁺ exchangers (NHE) is involved in the volume change through NaCl uptake. However, molecular mechanism of the NHE activation is unknown. In previous work, it has been indicated that Akt protein kinase activated by extracellular high concentration of NaCl is indispensable for RVI through NaCl uptake. In this study, we tried to identify other proteins phosphorylated by high concentration of NaCl and performed more detail investigation about Akt involvement in the NaCl uptake-dependent volume change under high concentration of NaCl.

To detect protein phosphorylation induced in the presence of high concentration of NaCl, 2-dimensional electrophoresis were performed. After purification of phospho-proteins, many proteins were newly phosphorylated under high concentration of NaCl. Of them, ERM proteins, a family of actin binding protein, was identified as proteins phosphorylated by high concentration of NaCl. Now characterization of the proteins was tried by using non-phosphorylated mutants. On the other hand, using RNAi method, it has been elucidated that Akt1 is involved in NaCl uptake in high concentration of NaCl. Moreover, intracellular pH imaging revealed that NHE activity is actually induced by Akt activity under NaCl high concentration conditions. Thus, it is concluded that many proteins are phosphorylated by high concentration of NaCl and that Akt activity is responsible for NHE activation by high NaCl concentration to induce NaCl uptake in HeLa cells.