The adaptor protein SHC1 is recruited to tyrosine-phosphorylated plasma membrane chloride channels

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Example

Protein kinase Syk was recently found to phosphorylate a specific tyrosine residue in three distinct disease-related chloride transport proteins: CFTR (in cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD)), NKCC2 and KCC3 (in kidney function and hypertension). Tyrosine phosphorylation downregulates the amount of CFTR present at the plasma membrane and a better understanding of this process may reveal novel therapeutic options for CF patients. Thus, we determined the adaptor proteins containing phospho-tyrosine-binding domains involved in the process. For their identification we used biotinylated synthetic peptides containing the phospho-tyrosines of each channel as baits and isolated adaptor proteins from physiologically relevant human and mouse cell lysates. After the peptide pull-down the samples were sent for mass spectrometry by Nano-LC-Triple TOF analysis to identify the obtained complex mixture of proteins. Following a bioinformatics analysis in order to choose the best candidates for experimental validation, we identified some proteins with phospho-tyrosine-binding domains together with proteins potentially involved in membrane trafficking. Using the peptide pull-down we validated that the adaptor protein SHC1 binds to NKCC2, KCC3 and CFTR peptides only in their tyrosine-phosphorylated form. We are currently investigating the interaction between CFTR and SHC1 and its impact on CFTR trafficking and plasma membrane anchoring. The results are expected to reveal the molecular mechanism underlying tyrosine-phosphorylation of these chloride channels and may suggest novel therapeutic targets for diseases like CF and hypertension.

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