Tyrosine phosphorylation modulates cell surface expression of chloride cotransporters NKCC2 and KCC3

Cláudia Almeida Loureiro[1,2], Patrícia Barros[1,2], Paulo Matos[1,2], Peter Jordan[1,2]

[1] Department of Human Genetics, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal

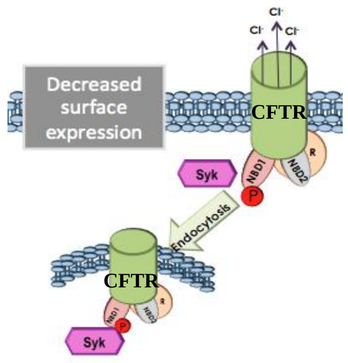
[2] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon.

Summary

Cellular chloride transport has a fundamental role in cell volume regulation and membrane potential formation, both in normal and tumor cells. Chloride entry or exit are mediated at the plasma membrane by cotransporter proteins of the solute carrier 12 family. For example, NKCC2 resorbs chloride with sodium and potassium at the apical membrane of epithelial cells in the kidney, whereas KCC3 releases chloride with potassium at the basolateral membrane. Although their ion transport activity is regulated by protein phosphorylation in response to signaling pathways, we describe an additional mechanism regulating the amount of cotransporter molecules inserted into the plasma membrane (PM). We show that protein kinase SYK phosphorylates the cotrans-porters NKCC2 and KCC3 at a specific tyrosine residue within their N-terminal cytoplasmic domains. This resulted in the modulation of their PM abundance, however, with opposite functional consequences: phosphorylation decreased NKCC2 but increased KCC3 levels at the PM.

Introduction

SYK was found to regulate CFTR surface abundance through phosphorylation at Tyr 512 in the NBD1 domain of CFTR



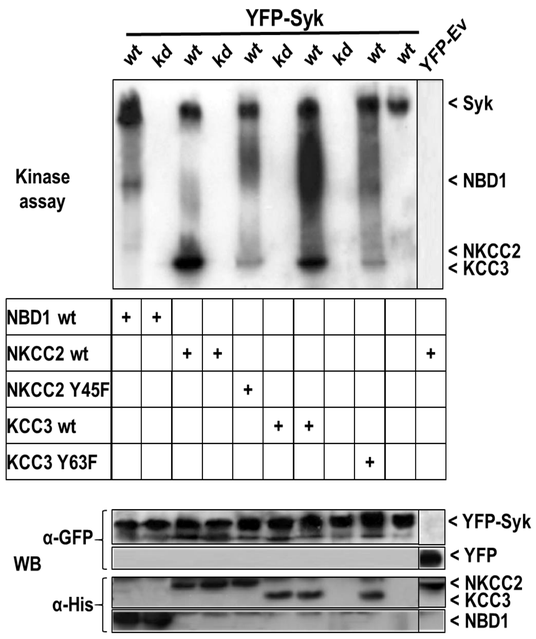
Mendes et al. (2011) Mol Cell Biol 31:4076-86

Analysis of the presence of a SYK recognition motif (Y-E/D-E/D-X) in 20 human ion channels or cotransporters involved in sodium, potassium or chloride transport

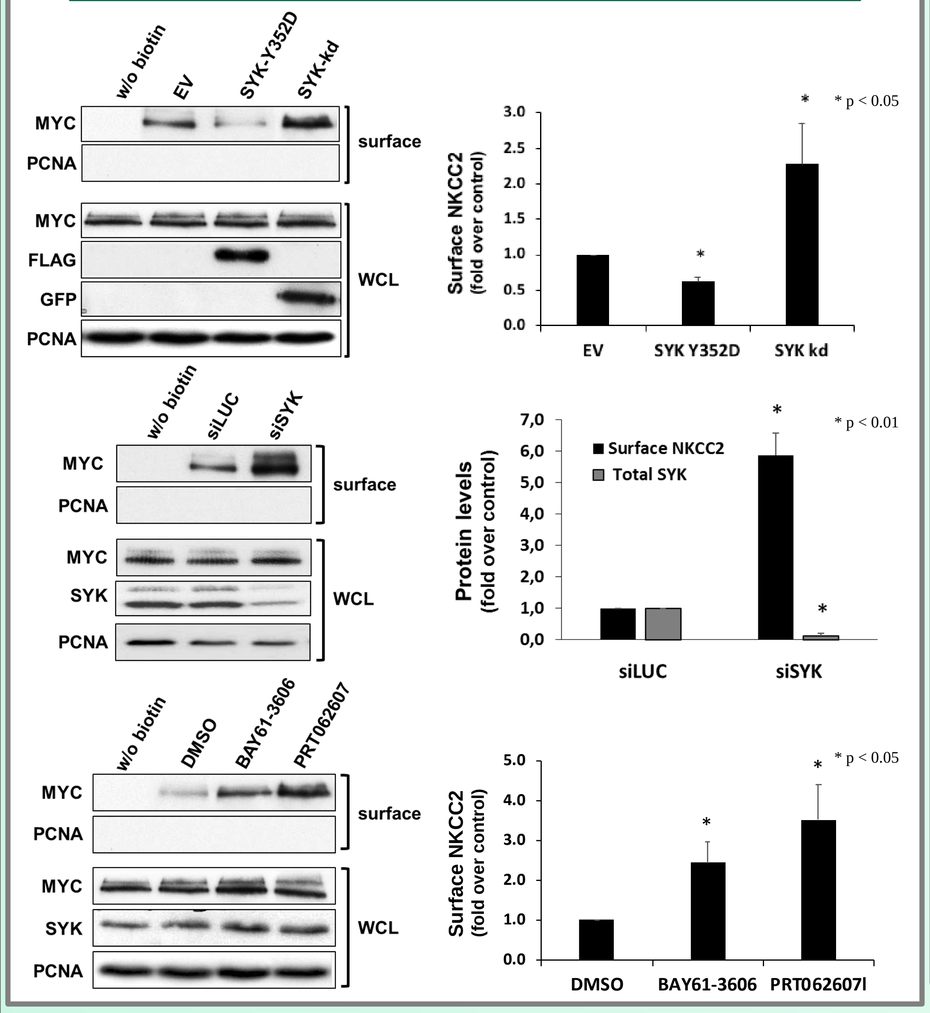
Transporter name	Gene	SYK motif
NKCC2	SLC12A1	Y45EET
NKCC1	SLC12A2	
NCC	SLC12A4	
KCC1	SLC12A3	
KCC2	SLC12A5	
KCC3	SLC12A6	Y63EEG
KCC4	SLC12A7	
Cl^-/HCO_3-exchanger	SLC26A3	
Pendrin	SLC26A4	
Pendrin L1	SLC26A6	
Sulfate anion transporter	SLC26A7	
Cl^-/HCO_3-exchanger	SLC26A9	
ROMK	KCNJ1	
ENaC-alpha	SCNN1A	
ENaC-beta	SCNN1B	
ENaC-gamma	SCNN1G	
ENaC-delta	SCNN1D	
TRPV4	TRPV4	
TRPV5	TRPV5	
TRPV6	TRPV6	
CFTR	CFTR	Y512DEY

Results

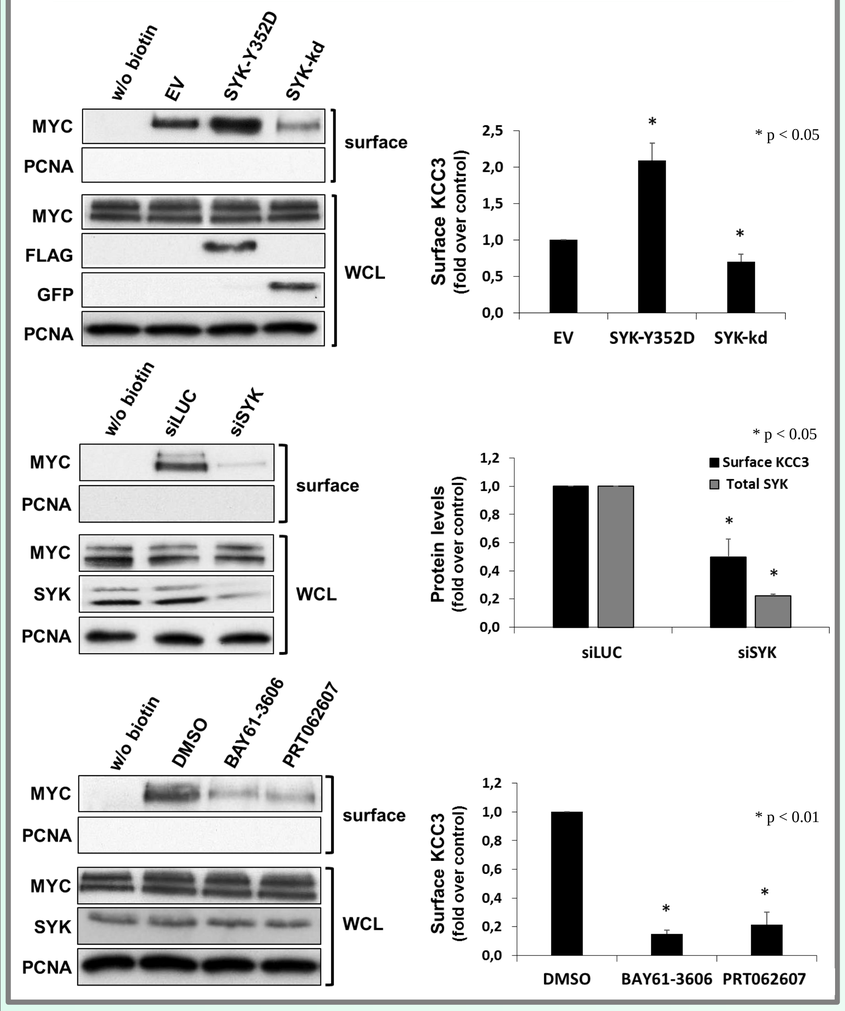
1. NKCC2 and KCC3 are *in vitro* substrates for SYK protein kinase



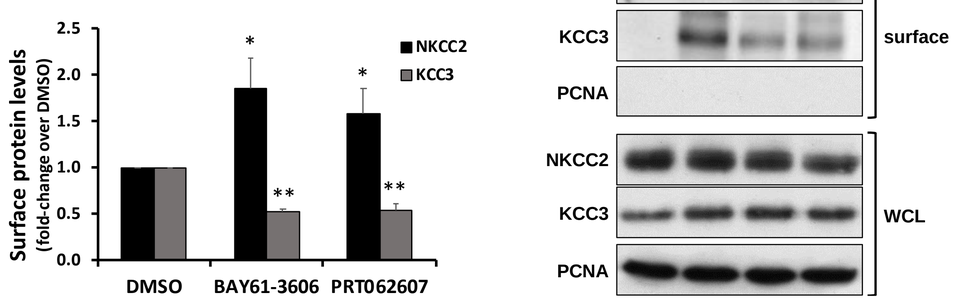
2. SYK activity decreases the plasma membrane expression of NKCC2 expressed in HEK293 cells



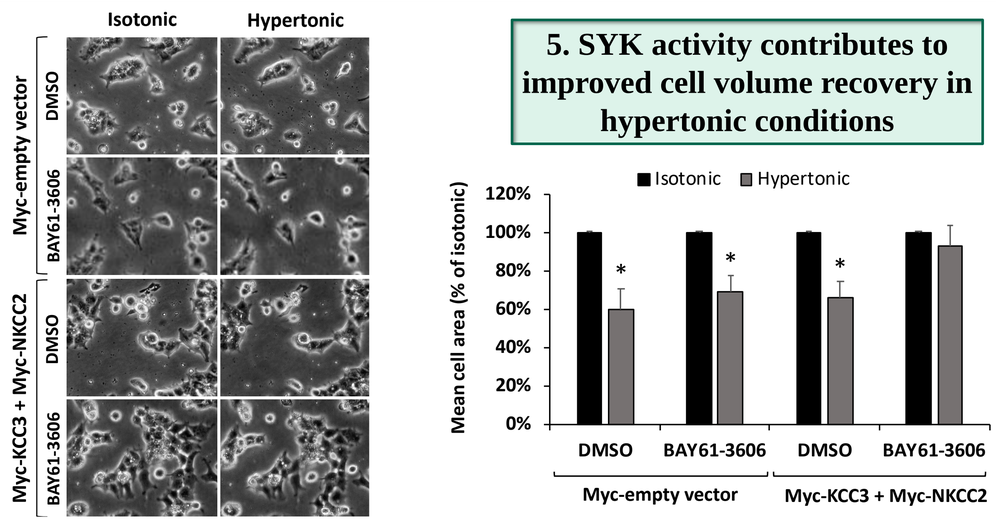
3. SYK activity increases the plasma membrane expression of KCC3 expressed in HEK293 cells



4. SYK activity modulates the plasma membrane expression of NKCC2 and KCC3 in opposite ways in the same cell



5. SYK activity contributes to improved cell volume recovery in hypertonic conditions



Conclusions

→ Phosphorylation by SYK on specific tyrosine residues modulates the plasma membrane levels of NKCC2 and KCC3, two key ion cotransporters involved in regulating renal electrolyte balance and cell volume.

→ Cells can apparently use the same mechanism to regulate NKCC2 and KCC3 in opposite ways.

Funding:

✓ UID/MULTI/04046/2019 (BioISI)
✓ PTDC/BIA-CEL/28408/2017
✓ Fellowship SFRH/BD/52488/2014 to CAL