WNK-SPAK/OSR1-NKCC1 Signaling Pathway in Ischemic Brain Edema and Injury

Dandan Sun
University of Pittsburgh
NKCC1 in $\text{Cl}^-_i$ Homeostasis and GABA Function

**a** High $[\text{Cl}^-_i]$ (immature)
- Depolarization
- $\text{K}^+$ flow
- $\text{Cl}^-$ entry
- $\text{GABA}_A$
- $\text{Na}^+/\text{K}^+$ pump
- NKCC1
- $2\text{Cl}^-$
- $\text{VDCC}$
- $\text{Ca}^{2+}$
- CLC2

**b** Low $[\text{Cl}^-_i]$ (mature)
- Hyperpolarization
- $\text{K}^+$ outflow
- $\text{Cl}^-$ exit
- $\text{GABA}_A$
- $\text{Na}^+/\text{K}^+$ pump
- NKCC1
- $2\text{Cl}^-$
- $\text{VDCC}$
- $\text{CLC2}$

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Inhibition of NKCC1 Reduces Ischemic Infarction

Yan et al., J Cereb Blood Flow Metab 2001

Chen et al., JCBFM 2005
Ion transporters in Ionic Dysregulation

Kahle KT et al., Physiology 2009

Boscia F et al., Glia 2016
Questions

➤ How NKCC1 is stimulated in ischemic brain?

➤ And what kinase signaling pathway stimulates NKCC1 phosphorylation and activation in ischemic brain?
Ion Transporter Kinases: The WNK-SPAK /OSR1 signaling pathway

Phospho-activation of NKCC1 Protein in Ischemic Brains

Begum et al., Stroke, 2015
Working Hypothesis

Diagram 1

Cerebral Ischemia

WNK3

SPAK

NKCC1

Disruption of Ionic Homeostasis

Grey/White Matter Injury

Neurological Deficit

Aim 1

Aim 2

Aim 3

Seth Alper, M.D., Ph.D.
Harvard University

Kristopher Kahle, M.D., Ph.D.
Yale University
WNK3 KO Mice Exhibit Reduced ADC Lesion Size in the MRI study

T. Kevin Hitchens, PhD

Hanshu Zhao, MD

Rachel Nepomuceno  Xin Gao, MD

Zhao et al., JCBF&M, 2016
Less severe ischemic infarct in SPAK HET and SPAK KO mice

Zhao et al., JCBF&M, 2016
Neurological function deficit tests after ischemic stroke

- Corner test
- Rotarod test
- Adhesive tape removal test
- Neurological score
Improved neurological function in WNK3 KO mice

Zhao et al., JCBF&M, 2016
Improved neurological function in SPAK HET and KO mice

Zhao et al., JCBF&M, 2016
WNK-Cab39-NKCC1 signaling in ischemic stroke of hypertensive rats
Selective Elevation of WNK Expression in SHRs after Ischemia

(a) Membrane

(b) Cytosol

(c) Membrane

(d) Cytosol

Iqbal Bhuiyan, Ph.D

Shanshan Song, MD

Arohan Subramanya, MD

Bhuiyan et al., JCBF&M, 2016
Developing inhibitors against the WNK-SPAK/OSR1 pathway
Anti-parasitic drug Closantel as a SPAK inhibitor in reducing infarct and hemispheric swelling

Iqbal Bhuiyan, Ph.D

Brad Molyneaux, MD, Ph.D

Bhuiyan, et al., unpublished
Novel WNK inhibitor WNK463 is not effective in reducing infarct and hemispheric swelling

Bhuiyan, et al., unpublished
Summary

- The evolutionarily-conserved WNK-SPAK/OSR1 kinase signaling pathway regulates the bumetanide-sensitive NKCC1 protein in ischemic brain.

- WNK3 function is associated with the pathophysiology of neuronal and oligodendrocyte injury following ischemic stroke.

- Our data highlight the WNK-SPAK/OSR1-NKCC1 signaling pathway as a novel, potential therapeutic targets for neuroprotection and cerebral edema following stroke.
Lab Members
Gulnaz Begum, PhD
M. Iqbal Bhuiyan, PhD
Karen Carney, PhD
Yan Yin, MD, PhD
Shanshan Song, MD, PhD
Victoria Pigott, BS
Nabiul Hasan, PhD
Xiudong Guan, MD
Eric Li, BS
Rachana Nayak
Abhishek Mishra

Collaborators
UW-Madison
  John Kuo, MD, PhD
  Pelin Cengiz, MD
  Peter Ferrazzano, MD

Harvard University
  Seth Alper, MD, PhD

Yale University
  Kris Kahle, MD, PhD

University of Cincinnati
  Gary Shull, PhD

Taiwan National Defense Medical Center
  Sung-Sen Yang, PhD
  Shih-Hua Lin, PhD

UC-San Francisco
  Ray Swanson, MD, PhD
  Anders Persson, PhD

Univ. of Pittsburgh
  Jun Chen, MD
  Elias Aizenman, PhD
  Edward Dixon, PhD
  Guodong Cao, PhD

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Thank You
Bumetanide Blunts Worsened Ischemic Injury in SHRs

**Protocol**
- Post-MCAO
- Saline or BMT (daily)
- Biochemical assays
- Neurological function tests

**Graph (b)**
- Comparison of rCBF (%) between WKY and SHR over time.

**Graph (d)**
- Infarct volume comparison between WKY and SHR.

**Graph (e)**
- Swelling volume comparison between WKY and SHR.

**Graph (f)**
- Latency to stand-off test between WKY and SHR.

**Graph (g)**
- Neurological score over time after reperfusion for WKY, WKY + BMT, SHR, and SHR + BMT.

*Bhuiyan et al., JCBF&M, 2016*
SPAK/OSR1 Phosphorylation and Degradation of tSPAK in SHRs after Ischemia

(a) Membrane

(b) Cytosol

(c) Membrane

Bhuiyan et al., JCBF&M, 2016
Up-regulation of Calcium-binding Protein 39 (Cab39)