Activation of K^+–Cl^-–cotransporter KCC2 by inhibiting the WNK-SPAK kinase signalling as a novel therapeutic strategy for epilepsy

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Abstract (300 word limit)

The Cl^--extruding transporter KCC2 (SLC12A5) critically modulates $GABA_A$ receptor signaling via its effect on neuronal Cl^- homeostasis. Previous studies have shown that KCC2 was downregulated in both epileptic patients and various epileptic animal models. We discovered that the in vitro dual phosphorylation of Thr906 and Thr1007 in the intracellular carboxyl (C)-terminal domain of KCC2, mediated by the Cl^--sensitive WNK-SPAK serine-threonine protein kinase complex, maintains the depolarizing action of GABA in immature neurons by antagonizing KCC2 Cl^- extrusion capacity. $GABA_AR$-mediated inhibition confines KCC2 to the plasma membrane, while antagonizing inhibition reduces KCC2 surface expression by increasing the lateral diffusion and endocytosis of the transporter. This mechanism utilizes Cl^- as an intracellular secondary messenger and is dependent on phosphorylation of KCC2 at threonines 906 and 1007 by the Cl^- -sensing kinase WNK1. We propose this mechanism contributes to the homeostasis of synaptic inhibition by rapidly adjusting neuronal $[Cl^-]_i$ to $GABA_AR$ activity. We further demonstrate here that this signaling pathway is rapidly and massively activated in an acute epilepsy model. This indicates that dephosphorylation of KCC2 at Thr906 and Thr1007 is a potent activator of KCC2 activity, and small molecular targets WNK-SAPK kinase signaling may be a novel therapeutic strategy for epilepsy.

Recent Publications (minimum 5)

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3. **Zhang J**, Bhuiyan MIH, Zhang T, Karimy J, Wu Z, Pigott VM, Zhang J, Huang H, Hassan MN, Skrzypiec AE, Mucha M, Duran D, Huang W, Pawlak R, Foley LM, Hitchens TK, Minnigh M, Poloyac S, Alper SL, Molyneaux BJ, Trevelyan A, Kahle K, Sun D, Deng X. Modulation of brain cation-Cl- cotransport via the SPAK kinase inhibitor ZT-1a. Nature Communications, 2020 Jan 7;11(1):78.
4. Heubl M, **Zhang J,** Pressey J, Renner M, Moutkine I, Russeau M, Eugène E, Kahle KT, Poncer JC and Lévi S. GABAA receptor dependent synaptic inhibition rapidly tunes KCC2 activity via the Cl−-sensitive WNK1 kinase. Nature Communications, 2017, 8 (1), 1776.
5. **Zhang J**, Gao G, Begum G, Wang J, Khanna AR, Shmukler BE, Daubner GM, de Los Heros P, Davies P, Varghese J, Bhuiyan MI, Duan J, Zhang J, Duran D, Alper SL, Sun D, Elledge SJ, Alessi DR, Kahle KT. Functional kinomics identifies a key regulatory module of swelling-regulated Cl- transport in the mammalian brain. Scientific Reports, 2016 6, 35986
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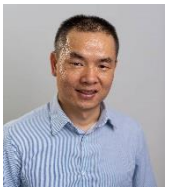
7. Pisella L, Gaiarsa JL, Diabira D, **Zhang J**, Khalilov I, Duan J, Kahle KT, Medina I. Impaired regulation of KCC2 phosphorylation leads to neuronal network dysfunction and neurodevelopmental pathology. Science Signalling, 2019 Oct 15;12(603). pii: eaay0300.
8. Tillman L, **Zhang J**. Crossing the Chloride Channel: The Current and Potential Therapeutic Value of the Neuronal K+-Cl- Cotransporter KCC2. BioMed Research International. 2019 May 21;2019:8941046.
9. Shekarabi M, **Zhang J**, Khanna AR, Ellison DH, Delpire E, Kahle KT. WNK kinase signaling in ion homeostasis and human disease. Cell Metabolism, 2017, Feb 7;25(2):285-299.
10. **Zhang J**, Karimy JK, Delpire E, Kahle KT. Targeting SPAK kinase in disorders of epithelial Cl- secretion. Expert Opinion On Therapeutic Targets, 2017 Aug;21(8):795-804.
11. Huang H, Song S, Banerjee S, Jiang T, **Zhang J**, Kahle KT, Sun D, Zhang Z. The WNK-SPAK/OSR1 kinases and the cation-chloride cotransporters as therapeutic targets for neurological diseases. Aging and Disease, 2019 Jun 1;10(3):626-636.
12. Alessi DR, **Zhang J**, Khanna A, Hochdörfer T, Shang Y, Kahle KT. The WNK-SPAK/OSR1 pathway: master regulator of Cation-Chloride Cotransporters. Science Signaling, 2014 Jul 15;7(334):re3.



Biography (150 word limit)

Dr Jinwei Zhang has a long track record of ground-breaking discovery in the field of cellular chloride homeostasis and cell volume regulation. He has published over 45 articles in peer-reviewed journals (with 20 first-author or corresponding author, total citations of 1800, h-index 22), including several in the highest impact journals, including Nature Medicine, Cell Metabolism, Neuron, and Nature Communications. Dr Zhang then made fundamental discoveries regarding the role of WNK-SPAK/OSR1-NKCC1/KCCs signalling pathway in Cl^- homeostasis through KCC2Thr906/1007 and NKCC1 Thr203/207/212 phosphorylation.

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Notes/Comments: