The Journal of Physiology

PERSPECTIVES

Beyond ion transport: KCC2 makes cells walk and talk

Anselm A. Zdebik
UCL Departments of Neuroscience, Physiology and Pharmacology and Medicine, University College London, London, UK

Email: a.zdebik@ucl.ac.uk

Tumour cells often express a repertoire of genes unusual for the cell lineage from which they originate. It is not surprising that these genes include ion channels and transporters, but because biophysicists are rarely interested in cancer biology, and scientists working on cancer often shy away from ion transport physiology, specific functions of these proteins in cancer cells tend to go unnoticed. One of the first observations of an ion channel's role in mammalian cancer was the surprising finding that *ether à go-go* (EAG) K^+ channels promote proliferation, abolish contact inhibition and increase tumour progression when inoculated in severe combined immunodeficiency (SCID, a common model for cancer research) mice (Pardo *et al.* 1999). In this case, the ion channel function appeared to confer malignancy to the cells. However, ion channels and transporters may also have functions unrelated to their role in ion transport. A well-investigated example is anion exchanger 1 (AE1), which anchors the cytoskeleton at the plasma membrane (Denker & Barber, 2002). Several mutations in AE1 lead to morphological abnormalities in erythrocytes, including hereditary spherocytosis.

In a recent issue of *The Journal of Physiology*, Wei *et al.* (2011) report that the potassium/chloride cotransporter KCC2 promotes cell migration in a human cervical cancer cell line. While Ellory and colleagues had shown that functional KCC cotransport, likely to be mediated by KCC1 and KCC3, promotes cancer growth and invasion in the same cell line (Shen *et al.* 2003), and that KCC3 activity impinges on cell cycle progression (Shen *et al.* 2001), Wei *et al.* (2011) now show that uterine cervical cancer cells express KCC2, which had previously been considered to be expressed in the central nervous system (CNS) exclusively (Williams *et al.* 1999) and that the stimulating effects of KCC2 on cell growth do not depend on its cotransport activity. These conclusions are based on a set of complementary experiments: Wei *et al.* (2011) first established that KCC2 is expressed in SiHa and HeLa cervical cancer cell lines, as well as in a breast cancer cell line and that KCC2 was expressed at the surface of SiHa cells, as shown by biotinylation. Manipulating the level of KCC2 expression did not affect cell proliferation, but induced marked morphological changes in SiHa cells. Lower KCC2 expression correlated with cells flattening out over a wider area in the dish and forming more focal adhesions. Consequently, migration and invasion were lower. Spreading, migration and invasion was enhanced by overexpression of wild-type KCC2 or a transport-deficient point mutant. Taken together these data suggest that while other KCC transporters affect tumour progression through their role as ion transporters, atypical KCC2 expression in these cells enhances malignancy via modulating the cytoskeleton.

KCC2, cloned in 1996, is considered a neuron-specific isoform of the potassium/chloride cotransporter family (Williams *et al.* 1999), comprising four members in mammals. KCC2 is expressed in mature neurones and renders fast postsynaptic inhibition hyperpolarizing, by coupling the Cl^- equilibrium potential to that of K^+ (Rivera *et al.* 1999). It thus counteracts the Na^+–K^+–$2Cl^-$ cotransporter NKCC1, accumulating Cl^- in immature neurones. However, apart from this developmentally important switch, a morphogenetic role for KCC2 in the developing CNS has emerged: Li *et al.* (2007) showed that dendritic spine development depends on KCC2 expression, that an N-terminal deletion mutant incapable of KCl cotransport is sufficient to correct the spine phenotype of KCC2-deficient neurones, and that KCC2 likely links to cytoskeleton via the neuronal isoform of protein 4.1, which has been implicated in coupling other transporters to the cytoskeleton (Denker & Barber, 2002). As an elegant control, Li *et al.* (2007) expressed a dominant-negative domain of 4.1, rendering spine morphology similar as in KCC2-deficient neurones. Moreover, they also showed that these morphological changes are reflected in altered excitatory synaptic transmission. This study established a transport-independent role for KCC2 in the maturation of excitatory synapses. Interestingly, another study has implicated expression of KCC2 in the generation of a stop signal for migrating interneurones (Bortone & Polleux, 2009). This effect was dependent on its cotransport activity and could therefore be mediated by altered intracellular calcium dynamics. As cervical cancer cell lines are not known to express $GABA_A$ or voltage-dependent Ca^{2+} channels, they are unlikely to respond in a similar manner.

How does KCC2 now make cervical cancer cells walk? The structural changes to the cytoskeleton described by Wei *et al.* (2011) are likely to change cell morphology and interaction with extracellular matrix molecules. This, in turn, may affect gene expression and change the overall repertoire of these cells to a more invasive phenotype. Further studies will have to show whether KCC2 links to the cytoskeleton via protein 4.1 in cells in which it is atypically expressed, and whether this is a universal mechanism enhancing malignancy of other cancer cell lines.

References

Bortone D & Polleux F (2009). *Neuron* **62**, 53–71.
Denker SP & Barber DL (2002). *Curr Opin Cell Biol* **14**, 214–220.
Li H, Khirug S, Cai C, Ludwig A, Blaesse P, Kolikova J, Afzalov R, Coleman SK, Lauri S, Airaksinen MS, Keinänen K, Khiroug L, Saarma M, Kaila K & Rivera C (2007). *Neuron* **56**, 1019–1033.
Pardo LA, del Camino D, Sanchez A, Alves F, Brüggemann A, Beckh S & Stühmer W (1999). *EMBO J* **18**, 5540–5547.
Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M & Kaila K (1999). *Nature* **397**, 251–255.
Shen MR, Chou CY, Hsu KF, Hsu YM, Chiu WT, Tang MJ, Alper SL & Ellory JC (2003). *J Biol Chem* **278**, 39941–39950.
Shen MR, Chou CY, Hsu KF, Liu HS, Dunham PB, Holtzman EJ & Ellory JC (2001). *Proc Natl Acad Sci U S A* **98**, 14714–14719.
Wei, W-C, Akerman, JC, Newey, SE, Pan, J, Clinch, NWV, Jacob, Y, Shen, M-R, Wilkins, RJ & Ellory, JC (2011). *J Physiol* **589**, 5349–5359.
Williams JR, Sharp JW, Kumari VG, Wilson M & Payne JA (1999). *J Biol Chem* **274**, 12656–12664.

 DOI: 10.1113/jphysiol.2011.221754