Original Paper

SPAK and OSR1 Sensitive Kir2.1 K⁺ Channels

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Key Words

Inwardly rectifying K⁺ channel • KCNJ2, oxidative stress-responsive kinase 1 • SPS1-related proline/alanine-rich kinase • WNK

Abstract

Background/Aims: Kir2.1 (KCNJ2) channels are expressed in neurons, skeletal muscle and cardiac tissue and maintain the resting membrane potential. The activity of those channels is regulated by diverse signalling molecules. The present study explored whether Kir2.1 channels are sensitive to the transporter and channels regulating kinases SPAK (SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress-responsive kinase 1), which are in turn regulated by WNK (with-no-K[Lys]) kinases. **Methods:** cRNA encoding Kir2.1 was injected into *Xenopus laevis* oocytes with or without additional injection of cRNA encoding wild-type SPAK, constitutively active ^{T233E}SPAK, WNK insensitive ^{T233A}SPAK, catalytically inactive ^{D212A}SPAK, wild-type OSR1, constitutively active ^{T185E}OSR1, WNK insensitive ^{T185A}OSR1 and catalytically inactive ^{D164A}OSR1. Inwardly rectifying K⁺ channel activity was quantified utilizing dual electrode voltage clamp and Kir2.1 channel protein abundance in the cell membrane was measured utilizing chemiluminescence of Kir2.1 containing an extracellular HA-tag epitope. **Results:** Kir2.1 activity was significantly enhanced by wild-type SPAK and ^{T233E}SPAK, but not by ^{T233A}SPAK and ^{D212A}SPAK, as well as by wild-type OSR1 and ^{T185E}OSR1, but not by ^{T185A}OSR1 and ^{D164A}OSR1. As shown for SPAK, the kinases enhanced Kir2.1 protein abundance in the cell membrane. The difference of current and conductance between oocytes expressing Kir2.1 together with SPAK or OSR1 and oocytes expressing Kir2.1 alone was dissipated following a 24 hours inhibition of channel insertion into the cell membrane by brefeldin A (5 μM). **Conclusions:** SPAK and OSR1 are both stimulators of Kir2.1 activity. They are presumably effective by enhancing channel insertion into the cell membrane.

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Introduction

The resting membrane potential in neurons, skeletal muscle and cardiac myocytes is maintained by inwardly rectifying K⁺ channels of the Kir2.x (*KCNJ*, IRK) subfamily [1], including the Kir2.1 isoform [2, 3]. Loss-of-function mutations of the gene encoding Kir2.1 lead to Andersen-Tawil syndrome (ATS), an inherited disease characterized by potassium-sensitive periodic paralysis, ventricular arrhythmias and dysmorphic features including syndactyly and altered face shapes [2, 4-7].

Kir2.1 activity is regulated by mitochondria [8, 9] and Kir2.1 channels are involved in accomplishment of ischemic preconditioning [10]. Further regulators of Kir2.1 channel activity include TNF- α [11], Chapsyn 110 [12], filamin-A [13], PSD95 [14], arachidonic acid [15], cholesterol [16], phosphatidylinositol 4,5-bisphosphate PI(4,5)P₂ [17-20], PKC [21], tyrosine kinases [22, 23], kinase-anchoring protein AKAP79 [24], Rho [25], as well as SAP97, CASK, Veli and Mint1 [26].

Powerful regulators of transporters and channels include SPAK (SPS1-related proline/alanine-rich kinase) [27-29] and OSR1 (oxidative stress-responsive kinase 1) [30, 31]. The kinases are regulated by WNK (with-no-K[Lys]) kinases [27, 32-35], kinases participating in the regulation of ion transport and blood pressure [36-40]. Genetic defects of WNK kinases may result in Gordon's syndrome, which is characterized by hypertension and hyperkalaemia [34, 35, 41, 42]. SPAK and OSR1 stimulate Na⁺, Cl⁻ (NCC) and Na⁺, K⁺, 2Cl⁻ (NKCC) co-transporters [30-33, 36, 43-52] and diverse ion channels [53-56]. By modifying cytosolic Cl⁻ concentration, the kinases influence neuronal excitability [57-59].

The present study explored, whether SPAK and/or OSR1 participate in the regulation of Kir2.1 channels. To this end, Kir2.1 was expressed in *Xenopus laevis* oocytes without or with additional expression of the respective wild-type, constitutively active or inactive kinases. In those oocytes, inwardly rectifying K⁺ current was determined utilizing dual electrode voltage clamp.

Materials and Methods

Ethical Statement

All experiments conform with the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg 1985) and were conducted according to the German law for the welfare of animals. Surgical procedures on the adult *Xenopus laevis* were reviewed and approved by the respective government authority of the state Baden-Württemberg (Regierungspräsidium) prior to the start of the study (Anzeige für Organentnahme nach 36).

Constructs

Constructs encoding human wild-type Kir2.1 (*KCNJ2*) [60], Kir2.1-HA containing an extracellular hemagglutinin epitope [61], human wild-type SPAK/pGHJ, constitutively active T^{233E}SPAK/pGHJ, WNK insensitive T^{233A}SPAK/pGHJ, catalytically inactive D^{212A}SPAK/pGHJ [32], human wild-type OSR1, constitutively active T^{185E}OSR1/pGHJ, WNK insensitive T^{185A}OSR1/pGHJ and catalytically inactive D^{164A}OSR1/pGHJ [62], were used for generation of cRNA as described previously [63, 64]. The constructs were a kind gift from Dario Alessi (University of Dundee).

Voltage clamp in *Xenopus* oocytes

Xenopus laevis oocytes were prepared as previously described [65, 66]. 10 ng cRNA encoding Kir2.1 and/or 10 ng of cRNA encoding wild-type, constitutively active or inactive kinases were injected on the same day after preparation of the oocytes [67, 68]. The oocytes were maintained at 17°C in ND96-A, a solution containing (in mM): 88.5 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, 5 HEPES/NaOH (pH 7.4), 5 sodium pyruvate (C₃H₃NaO₃), Gentamycin (100 mg/l), Tetracycline (50 mg/l), Ciprofloxacin (1.6 mg/l), and Theophiline (90 mg/l) [64, 69]. The voltage clamp experiments were performed at room temperature 3 days after the first

injection. Kir2.1 channel currents were elicited every 20 s with 1 s pulses from -150 mV to +30 mV applied from a holding potential of -60 mV. The data were filtered at 2 kHz and recorded with a Digidata A/D-D/A converter (1322A Axon Instruments) and Clampex 9.2 software for data acquisition and analysis (Axon Instruments) [70-72]. The control superfusate (ND96-B) contained (in mM): 93.5 NaCl, 5 KCl, 1.8 CaCl₂, 1 MgCl₂ and 5 HEPES/NaOH (pH 7.4). The flow rate of the superfusion was approx. 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s [73-75].

Detection of Kir2.1-HA cell surface expression by chemiluminescence

To determine Kir2.1-HA cell surface expression by chemiluminescence, *Xenopus* oocytes were incubated with monoclonal anti-HA antibody conjugated to Horseradish Peroxidase (diluted 1:500, Miltenyi Biotec, Germany). Individual oocytes were placed in 96 well plates with 20 µl of SuperSignal ELISA Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL, USA) and chemiluminescence of single oocytes was quantified in a luminometer (Walter Wallac 2 plate reader, Perkin Elmer, Jügesheim, Germany) by integrating the signal over a period of 1 s [76]. Results display normalized relative light units [77, 78].

Statistical analysis

Data are provided as means ± SEM, n represents the number of oocytes investigated. As different batches of oocytes may yield different results, comparisons were always made within a given oocyte batch. All voltage clamp experiments were repeated with at least 3 batches of oocytes; in all repetitions qualitatively similar data were obtained. Data were tested for significance using ANOVA (Tukey test or Kruskal-Wallis test) or the unpaired students t-test, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

The present study explored whether the transport regulating kinases SPAK (SPS1-related proline/alanine-rich kinase) and/or OSR1 (oxidative stress-responsive kinase 1) modify the activity of the inwardly rectifying Kir2.1 K⁺ channels. To this end, cRNA encoding Kir2.1 was injected into *Xenopus laevis* oocytes with or without additional injection of cRNA encoding the respective kinases. The K⁺ currents in those oocytes were determined by dual electrode voltage clamp experiments.

As shown in Fig. 1, inwardly rectifying K⁺ currents were negligible in water-injected oocytes or in oocytes expressing SPAK alone, indicating that the oocytes did not express appreciable inwardly rectifying K⁺ channels. In contrast, large inwardly rectifying K⁺ currents were observed in oocytes expressing Kir2.1. The additional co-expression of wild-type SPAK was followed by a significant increase of the inwardly rectifying K⁺ currents.

In order to test, whether kinase activity was required for the effect of SPAK, cRNA encoding Kir2.1 was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding constitutively active T233E SPAK or cRNA encoding WNK insensitive T233A SPAK, or cRNA encoding catalytically inactive D212A SPAK. As shown in Fig. 2, the inwardly rectifying K⁺ currents in Kir2.1 expressing oocytes was significantly increased by co-expression of T233E SPAK, but neither by co-expression of T233A SPAK, nor by co-expression of D212A SPAK.

The increase of inwardly rectifying current following co-expression of SPAK could have been secondary to increase of channel protein abundance in the cell membrane. Thus, chemiluminescence was employed in order to test whether the effect of SPAK on Kir2.1 currents was paralleled by an effect of the kinase on Kir2.1 protein abundance in the cell membrane. As illustrated in Fig. 3, the cell surface expression of the Kir2.1 channel protein in *Xenopus* oocytes injected with cRNA encoding tagged Kir2.1-HA was indeed increased by the co-expression of wild-type SPAK.

The influence of SPAK on Kir2.1 channel activity could have resulted from accelerated insertion of channel protein into the cell membrane or from delayed retrieval of channel protein from the cell membrane. In order to discriminate between those two possibilities, the insertion of new channel protein into the cell membrane was inhibited by brefeldin A

Fig. 1. Co-expression of wild-type SPAK increased the inwardly rectifying K^+ current in Kir2.1-expressing *Xenopus* oocytes. **A:** Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing wild-type SPAK alone (b), expressing Kir2.1 alone (c) or expressing Kir2.1 with additional co-expression of wild-type SPAK (d). The voltage protocol is shown (not to scale). **B:** Arithmetic means $\pm$ SEM ($n = 9-37$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles), expressing SPAK alone (black triangles), expressing Kir2.1 alone (white squares) or expressing Kir2.1 with SPAK (black squares). **C, D:** Arithmetic means $\pm$ SEM ($n = 9-37$) of (C) maximal current and (D) conductance calculated by linear fit of I/V -curves shown in B between -150 mV and -120 mV in *Xenopus* oocytes injected with water (dotted bar), expressing SPAK alone (grey bar) or expressing Kir2.1 without (white bar) or with (black bar) additional co-expression of wild-type SPAK. ** ($p < 0.01$), *** ($p < 0.001$) indicate statistically significant difference from oocytes expressing Kir2.1 alone.

(5 μM). A 24 hours incubation with brefeldin A was followed by a decline of Kir2.1 channel activity pointing to retrieval of channel protein from the cell membrane. As illustrated in Fig. 4, the treatment was followed by a decline of inwardly rectifying K^+ current to similarly low values in oocytes expressing Kir2.1 alone and in oocytes expressing both, Kir2.1 and SPAK. Thus, co-expression of SPAK did not delay the retrieval of channel protein from the cell membrane.

A similar series of experiments was performed to uncover effects of OSR1 on Kir2.1 channel activity. As shown in Fig. 5, the inwardly rectifying K^+ current was significantly higher in Kir2.1 expressing oocytes co-expressing wild-type OSR1 than in oocytes expressing Kir2.1 alone.

Again, mutant kinases were tested to explore whether the effect of OSR1 required kinase activity. As illustrated in Fig. 6, the effect of wild-type OSR1 on the inwardly rectifying K^+ current in Kir2.1 expressing oocytes was mimicked by constitutively active ^{T185E}OSR1 but neither by the WNK insensitive inactive ^{T185A}OSR1 nor by the catalytically dead ^{D164A}OSR1 (Fig. 5).

Fig. 2. The effect of wild-type SPAK on Kir2.1 was mimicked by constitutively active $T233E$ SPAK but not by WNK insensitive $T233A$ SPAK or catalytically inactive $D212A$ SPAK. **A:** Representative original tracings showing currents in *Xenopus* oocytes injected with water (a) expressing Kir2.1 alone (b), expressing Kir2.1 together with constitutively active $T233E$ SPAK (c), with WNK insensitive $T233A$ SPAK (d) or with catalytically inactive $D212A$ SPAK (e). **B:** Arithmetic means $\pm$ SEM ($n = 13-33$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles), expressing Kir2.1 alone (white squares) or expressing Kir2.1 together with constitutively active $T233E$ SPAK (black squares), inactive $T233A$ SPAK (grey diamonds) or catalytically inactive $D212A$ SPAK (black triangles). **C, D:** Arithmetic means $\pm$ SEM ($n = 13-33$) of (C) maximal current and (D) conductance calculated by linear fit of I/V -curves shown in B between -150 mV and -120 mV in *Xenopus* oocytes injected with water (dotted bar), expressing Kir2.1 alone (white bar), expressing Kir2.1 with constitutively active $T233E$ SPAK (black bar), or expressing Kir2.1 with inactive $T233A$ SPAK (light grey bar), or $D212A$ SPAK (dark grey bar). *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing Kir2.1 alone.

Fig. 3. Co-expression of SPAK increased the Kir2.1 protein abundance within the plasma membrane of *Xenopus* oocytes. Arithmetic means $\pm$ SEM ($n = 84-88$) from the normalized data of Kir2.1-HA protein abundance as determined by chemiluminescence in the plasma membrane of *Xenopus* oocytes injected with water (dotted bar) or expressing Kir2.1-HA without (white bar) or with (black bar) additional expression of wild-type SPAK. *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing Kir2.1-HA alone.

As illustrated in Fig. 7, brefeldin A treatment again decreased the inwardly rectifying K^+ current to similarly low values in oocytes expressing Kir2.1 alone and in oocytes expressing both, Kir2.1 and OSR1. Thus, co-expression of OSR1 did not delay the retrieval of channel protein from the cell membrane.

Fig. 4. Effect of brefeldin A on inwardly rectifying K⁺ current in Kir2.1 and SPAK expressing oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes expressing Kir2.1 alone (a,b) or expressing Kir2.1 together with wild type SPAK (c,d), prior to (a,c) and following (b,d) a 24 hours incubation with 5 μM brefeldin A. B: Arithmetic means ± SEM (n = 14-21) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus laevis* oocytes expressing Kir2.1 alone (white square) or expressing Kir2.1 together with OSR1 (black squares), prior to (white circles) and following (black circles) a 24 hours incubation with 5 μM brefeldin A. C, D: Arithmetic means ± SEM (n = 14-21) of (C) maximal current and (D) conductance calculated by linear fit of respective I/V-curves between -150 mV and -120 mV in *Xenopus* oocytes expressing Kir2.1 alone (white bars) or expressing Kir2.1 together with wild type SPAK (black bars) prior to (left bars, 0h) and following a 24 hours incubation with 5 μM brefeldin A (right bars). * (p<0.05), ** (p<0.01) indicates statistically significant difference from oocytes expressing Kir2.1 alone, ### (p<0.001) indicates statistically significant difference from the respective oocytes prior to brefeldin A treatment.

Discussion

The present study discloses SPAK/OSR1 sensitivity of Kir2.1 K⁺ channels. Co-expression of either SPAK or OSR1 increases the Kir2.1 channel activity and enhances the respective K⁺ current in Kir2.1 expressing oocytes. As shown for SPAK, the effect is at least partially due to increase of channel protein abundance in the cell membrane. In theory, the effect could have been due to increased transport to the cell membrane, stabilization of the protein in the cell membrane or inhibition of internalization from the cell membrane. Inhibition of channel insertion into the cell membrane by brefeldin A dissipates, however, the effect of the kinases, indicating that SPAK/OSR1 do not appreciably stabilize the inserted protein or delay internalization of the channel protein. This observation is suggestive for an effect of SPAK/OSR1 on channel trafficking and/or insertion of channel protein into the cell membrane. As experiments have been performed in oocytes injected with cRNA encoding Kir2.1, the observed effects do not reflect stimulation of Kir2.1 transcription.

Fig. 5. Co-expression of wild-type OSR1 increased the inwardly rectifying K⁺ current in Kir2.1-expressing *Xenopus* oocytes. **A:** Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing wild-type OSR1 alone (b), expressing Kir2.1 alone (c) or expressing Kir2.1 with additional co-expression of wild-type OSR1 (d). The voltage protocol is shown (not to scale). **B:** Arithmetic means $\pm$ SEM ($n = 9-35$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles), expressing OSR1 alone (black triangle), expressing Kir2.1 alone (white squares) or expressing Kir2.1 with OSR1 (black squares). **C, D:** Arithmetic means $\pm$ SEM ($n = 9-35$) of (C) maximal current and (D) conductance calculated by linear fit of I/V -curves shown in B between -150 mV and -120 mV in *Xenopus* oocytes injected with water (dotted bar), expressing OSR1 alone (grey bar) or expressing Kir2.1 without (white bar) or with (black bar) additional co-expression of wild-type OSR1. *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing Kir2.1 alone.

The effect of wild type SPAK is mimicked by constitutively active ^{T233E}SPAK but not by WNK insensitive ^{T233A}SPAK or catalytically inactive ^{D212A}SPAK. Similarly, the effect of wild-type OSR1 is mimicked by constitutively active ^{T185E}OSR1, but not by WNK insensitive ^{T185A}OSR1 or catalytically inactive ^{D164A}OSR1. To become effective, SPAK and OSR1 thus require activation by WNK kinases and catalytic activity of the enzymes. This observation does not rule out that the kinases are effective by phosphorylating regulators of the Kir2.1 channel protein thus indirectly modifying channel activity.

K⁺ channel activity is a decisive determinant of cell volume [79]. The kinases could thus influence Kir2.1 channel activity indirectly by modifying cell volume. SPAK and OSR1 are both powerful stimulators of the NaCl co-transporter and the Na⁺,K⁺,2Cl⁻ co-transporter and powerful inhibitors of KCl co-transporters, effects collectively increasing cellular K⁺ uptake [30-33, 36, 43-52]. Cellular accumulation of KCl with osmotically obliged water is followed by cell swelling [80-82]. Activation of Kir2.1 channels may result in blunting of cellular KCl uptake and cell swelling.

The present study does not allow safe conclusions as to the functional significance of SPAK and OSR1 sensitive Kir2.1 activity. Heteromeric Kir2.1 channels generate the cardiac

Fig. 6. The effect of wild type OSR1 on Kir2.1 was mimicked by constitutively active $T185^E$ OSR1 but not by WNK insensitive $T185^A$ OSR1 or catalytically inactive $D164^A$ OSR1. **A:** Representative original tracings showing currents in *Xenopus* oocytes injected with water (a) expressing Kir2.1 alone (b), expressing Kir2.1 together with constitutively active $T185^E$ OSR1 (c), with WNK insensitive $T185^A$ OSR1 (d) or with catalytically inactive $D164^A$ OSR1 (e). **B:** Arithmetic means $\pm$ SEM ($n = 9-35$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus laevis* oocytes injected with water (white circles), expressing Kir2.1 alone (white squares) or expressing Kir2.1 together with constitutively active $T185^E$ OSR1 (black squares), inactive $T185^A$ OSR1 (grey diamonds) or catalytically inactive $D164^A$ OSR1 (black triangle). **C, D:** Arithmetic means $\pm$ SEM ($n = 17-35$) of (C) maximal current and (D) conductance calculated by linear fit of I/V -curves shown in B between -150 mV and -120 mV in *Xenopus* oocytes injected with water (dotted bar), expressing Kir2.1 alone (white bar), expressing Kir2.1 with constitutively active $T185^E$ OSR1 (black bar), or expressing Kir2.1 with inactive $T185^A$ OSR1 (light grey bar), or $D164^A$ OSR1 (dark grey bar). ** ($p < 0.01$) indicates statistically significant difference from oocytes expressing Kir2.1 alone.

inwardly rectifying K^+ current I_{K1} [83-85] and Kir2.1 gain-of-function mutations cause atrial fibrillation [84, 86].

The present observations may be relevant for neurobiology. Kir2.1 channels are expressed in neurons [87-90] and glial cells [91-99] and contribute to spatial K^+ buffering thus influencing neuronal excitability [87, 94, 100]. The ubiquitously expressed SPAK [101] and OSR1 similarly influence neuronal excitability, an effect, however, attributed to their effect on cytosolic Cl^- concentration in neurons, which is critically important for the effect of Cl^- channel opening on cell membrane potential and thus excitation [57-59]. Kir2.1 is further expressed in retinal Müller cells [102-107] and deranged function of Kir2.1 in those cells contributes to the pathophysiology of uveitis [102]. Moreover, Kir2.1 has been implicated in neurovascular coupling [108]. Defective Kir2.1 accounts for the Andersen-Tawil syndrome [2, 4-7], a genetic disease leading to several disorders including potassium-sensitive periodic paralysis. Clearly, additional experimental effort is required to elucidate whether or not SPAK/OSR1 sensitive regulation of Kir2.1 is of neurobiological relevance.

In conclusion, SPAK and OSR1 both moderately up-regulate Kir2.1 mediated inwardly rectifying current, an effect possibly participating in the regulation of diverse functions including cell volume regulation and excitability.

Fig. 7. Effect of brefeldin A on inwardly rectifying K⁺ current in Kir2.1 and OSR1 expressing oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes expressing Kir2.1 alone (a,b) or expressing Kir2.1 together with wild type OSR1 (c,d), prior to (a,c) and following (b,d) a 24 hours incubation with 5 μ M brefeldin A. B: Arithmetic means $\pm$ SEM (n = 14-20) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus laevis* oocytes expressing Kir2.1 alone (white squares) or expressing Kir2.1 together with OSR1 (black squares), prior to (white circles) and following (black circles) a 24 hours incubation with 5 μ M brefeldin A. C, D: Arithmetic means $\pm$ SEM (n = 14-20) of (C) maximal current and (D) conductance calculated by linear fit of respective I/V-curves between -150 mV and -120 mV in *Xenopus* oocytes expressing Kir2.1 alone (white bars) or expressing Kir2.1 together with wild type OSR1 (black bars) prior to (left bars, 0h) and following a 24 hours incubation with 5 μ M brefeldin A (right bars). * ($p < 0.05$), ** ($p < 0.01$) indicates statistically significant difference from oocytes expressing Kir2.1 alone, ### ($p < 0.001$) indicates statistically significant difference from the respective oocytes prior to brefeldin A treatment.

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Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

References

- 1 Lopatin AN, Nichols CG: Inward rectifiers in the heart: an update on I(K1). *J Mol Cell Cardiol* 2001;33:625-638.
- 2 Jongsma HJ, Wilders R: Channelopathies: Kir2.1 mutations jeopardize many cell functions. *Curr Biol* 2001;11:R747-750.
- 3 Bates EA: A potential molecular target for morphological defects of fetal alcohol syndrome: Kir2.1. *Curr Opin Genet Dev* 2013;23:324-329.
- 4 Andersen ED, Krasilnikoff PA, Overvad H: Intermittent muscular weakness, extrasystoles, and multiple developmental anomalies. A new syndrome? *Acta Paediatr Scand* 1971;60:559-564.
- 5 Plaster NM, Tawil R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, Donaldson MR, Iannaccone ST, Brunt E, Barohn R, Clark J, Deymeer F, George AL, Jr., Fish FA, Hahn A, Nitu A, Ozdemir C, Serdaroglu P, Subramony SH, Wolfe G, Fu YH, Ptacek LJ: Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* 2001;105:511-519.
- 6 Sansone V, Griggs RC, Meola G, Ptacek LJ, Barohn R, Iannaccone S, Bryan W, Baker N, Janas SJ, Scott W, Ririe D, Tawil R: Andersen's syndrome: a distinct periodic paralysis. *Ann Neurol* 1997;42:305-312.
- 7 Tawil R, Ptacek LJ, Pavlakis SG, DeVivo DC, Penn AS, Ozdemir C, Griggs RC: Andersen's syndrome: potassium-sensitive periodic paralysis, ventricular ectopy, and dysmorphic features. *Ann Neurol* 1994;35:326-330.
- 8 Collins A, Larson M: Differential sensitivity of inward rectifier K⁺ channels to metabolic inhibitors. *J Biol Chem* 2002;277:35815-35818.
- 9 Lodge NJ, Normandin DE: Alterations in Ito1, IKr and Ik1 density in the BIO TO-2 strain of syrian myopathic hamsters. *J Mol Cell Cardiol* 1997;29:3211-3221.
- 10 Diaz RJ, Zobel C, Cho HC, Batthish M, Hinek A, Backx PH, Wilson GJ: Selective inhibition of inward rectifier K⁺ channels (Kir2.1 or Kir2.2) abolishes protection by ischemic preconditioning in rabbit ventricular cardiomyocytes. *Circ Res* 2004;95:325-332.
- 11 Vicente R, Coma M, Busquets S, Moore-Carrasco R, Lopez-Soriano FJ, Argiles JM, Felipe A: The systemic inflammatory response is involved in the regulation of K(+) channel expression in brain via TNF-alpha-dependent and -independent pathways. *FEBS Lett* 2004;572:189-194.
- 12 Leyland ML, Dart C: An alternatively spliced isoform of PSD-93/chapsyn 110 binds to the inwardly rectifying potassium channel, Kir2.1. *J Biol Chem* 2004;279:43427-43436.
- 13 Sampson LJ, Leyland ML, Dart C: Direct interaction between the actin-binding protein filamin-A and the inwardly rectifying potassium channel, Kir2.1. *J Biol Chem* 2003;278:41988-41997.
- 14 Nehring RB, Wischmeyer E, Doring F, Veh RW, Sheng M, Karschin A: Neuronal inwardly rectifying K(+) channels differentially couple to PDZ proteins of the PSD-95/SAP90 family. *J Neurosci* 2000;20:156-162.
- 15 Liu Y, Liu D, Heath L, Meyers DM, Krafte DS, Wagoner PK, Silvia CP, Yu W, Curran ME: Direct activation of an inwardly rectifying potassium channel by arachidonic acid. *Mol Pharmacol* 2001;59:1061-1068.
- 16 Romanenko VG, Fang Y, Byfield F, Travis AJ, Vandenberg CA, Rothblat GH, Levitan I: Cholesterol sensitivity and lipid raft targeting of Kir2.1 channels. *Biophys J* 2004;87:3850-3861.
- 17 Donaldson MR, Jensen JL, Tristani-Firouzi M, Tawil R, Bendahhou S, Suarez WA, Cobo AM, Poza JJ, Behr E, Wagstaff J, Szepietowski P, Pereira S, Mozaffar T, Escolar DM, Fu YH, Ptacek LJ: PIP2 binding residues of Kir2.1 are common targets of mutations causing Andersen syndrome. *Neurology* 2003;60:1811-1816.
- 18 Rohacs T, Chen J, Prestwich GD, Logothetis DE: Distinct specificities of inwardly rectifying K(+) channels for phosphoinositides. *J Biol Chem* 1999;274:36065-36072.
- 19 Soom M, Schonherr R, Kubo Y, Kirsch C, Klinger R, Heinemann SH: Multiple PIP2 binding sites in Kir2.1 inwardly rectifying potassium channels. *FEBS Lett* 2001;490:49-53.
- 20 Xiao J, Zhen XG, Yang J: Localization of PIP2 activation gate in inward rectifier K⁺ channels. *Nat Neurosci* 2003;6:811-818.
- 21 Zitron E, Kiesecker C, Luck S, Kathofer S, Thomas D, Kreye VA, Kiehn J, Katus HA, Schoels W, Karle CA: Human cardiac inwardly rectifying current IKir2.2 is upregulated by activation of protein kinase A. *Cardiovasc Res* 2004;63:520-527.
- 22 Henry P, Pearson WL, Nichols CG: Protein kinase C inhibition of cloned inward rectifier (HRK1/KIR2.3) K⁺ channels expressed in *Xenopus* oocytes. *J Physiol* 1996;495:681-688.

- 23 Wischmeyer E, Doring F, Karschin A: Acute suppression of inwardly rectifying Kir2.1 channels by direct tyrosine kinase phosphorylation. *J Biol Chem* 1998;273:34063-34068.
- 24 Dart C, Leyland ML: Targeting of an A kinase-anchoring protein, AKAP79, to an inwardly rectifying potassium channel, Kir2.1. *J Biol Chem* 2001;276:20499-20505.
- 25 Jones SV: Role of the small GTPase Rho in modulation of the inwardly rectifying potassium channel Kir2.1. *Mol Pharmacol* 2003;64:987-993.
- 26 Leonoudakis D, Conti LR, Radeke CM, McGuire LM, Vandenberg CA: A multiprotein trafficking complex composed of SAP97, CASK, Veli, and Mint1 is associated with inward rectifier Kir2 potassium channels. *J Biol Chem* 2004;279:19051-19063.
- 27 Rafiqi FH, Zuber AM, Glover M, Richardson C, Fleming S, Jovanovic S, Jovanovic A, O'Shaughnessy KM, Alessi DR: Role of the WNK-activated SPAK kinase in regulating blood pressure. *EMBO Mol Med* 2010;2:63-75.
- 28 Castaneda-Bueno M, Gamba G: SPAKling insight into blood pressure regulation. *EMBO Mol Med* 2010;2:39-41.
- 29 Yang SS, Lo YF, Wu CC, Lin SW, Yeh CJ, Chu P, Sytwu HK, Uchida S, Sasaki S, Lin SH: SPAK-knockout mice manifest Gitelman syndrome and impaired vasoconstriction. *J Am Soc Nephrol* 2010;21:1868-1877.
- 30 Lin SH, Yu IS, Jiang ST, Lin SW, Chu P, Chen A, Sytwu HK, Sohara E, Uchida S, Sasaki S, Yang SS: Impaired phosphorylation of Na(+)-K(+)-2Cl(-) cotransporter by oxidative stress-responsive kinase-1 deficiency manifests hypotension and Bartter-like syndrome. *Proc Natl Acad Sci USA* 2011;108:17538-17543.
- 31 Villa F, Deak M, Alessi DR, van Aalten DM: Structure of the OSR1 kinase, a hypertension drug target. *Proteins* 2008;73:1082-1087.
- 32 Vitari AC, Deak M, Morrice NA, Alessi DR: The WNK1 and WNK4 protein kinases that are mutated in Gordon's hypertension syndrome phosphorylate and activate SPAK and OSR1 protein kinases. *Biochem J* 2005;391:17-24.
- 33 Vitari AC, Thastrup J, Rafiqi FH, Deak M, Morrice NA, Karlsson HK, Alessi DR: Functional interactions of the SPAK/OSR1 kinases with their upstream activator WNK1 and downstream substrate NKCC1. *Biochem J* 2006;397:223-231.
- 34 Glover M, Zuber AM, O'Shaughnessy KM: Hypertension, dietary salt intake, and the role of the thiazide-sensitive sodium chloride transporter NCCT. *Cardiovasc Ther* 2011;29:68-76.
- 35 O'Reilly M, Marshall E, Speirs HJ, Brown RW: WNK1, a gene within a novel blood pressure control pathway, tissue-specifically generates radically different isoforms with and without a kinase domain. *J Am Soc Nephrol* 2003;14:2447-2456.
- 36 Kahle KT, Rinehart J, Lifton RP: Phosphoregulation of the Na-K-2Cl and K-Cl cotransporters by the WNK kinases. *Biochim Biophys Acta* 2010;1802:1150-1158.
- 37 Flatman PW: Cotransporters, WNKs and hypertension: an update. *Curr Opin Nephrol Hypertens* 2008;17:186-192.
- 38 Furgeson SB, Linas S: Mechanisms of type I and type II pseudohypoaldosteronism. *J Am Soc Nephrol* 2010;21:1842-1845.
- 39 Uchida S: Pathophysiological roles of WNK kinases in the kidney. *Pflugers Arch* 2010;460:695-702.
- 40 Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinases. *Science* 2001;293:1107-1112.
- 41 Achard JM, Disse-Nicodeme S, Fiquet-Kempf B, Jeunemaitre X: Phenotypic and genetic heterogeneity of familial hyperkalaemic hypertension (Gordon syndrome). *Clin Exp Pharmacol Physiol* 2001;28:1048-1052.
- 42 Capasso G, Cantone A, Evangelista C, Zaccchia M, Trepiccone F, Acone D, Rizzo M: Channels, carriers, and pumps in the pathogenesis of sodium-sensitive hypertension. *Semin Nephrol* 2005;25:419-424.
- 43 Delpire E, Gagnon KB: SPAK and OSR1, key kinases involved in the regulation of chloride transport. *Acta Physiol (Oxf)* 2006;187:103-113.
- 44 Delpire E, Gagnon KB: SPAK and OSR1: STE20 kinases involved in the regulation of ion homeostasis and volume control in mammalian cells. *Biochem J* 2008;409:321-331.
- 45 Gimenez I: Molecular mechanisms and regulation of furosemide-sensitive Na-K-Cl cotransporters. *Curr Opin Nephrol Hypertens* 2006;15:517-523.

- 46 Richardson C, Sakamoto K, de los HP, Deak M, Campbell DG, Prescott AR, Alessi DR: Regulation of the NKCC2 ion cotransporter by SPAK-OSR1-dependent and -independent pathways. *J Cell Sci* 2011;124:789-800.
- 47 Gagnon KB, Delpire E: On the substrate recognition and negative regulation of SPAK, a kinase modulating Na⁺-K⁺-2Cl⁻ cotransport activity. *Am J Physiol Cell Physiol* 2010;299:C614-C620.
- 48 Glover M, O'Shaughnessy KM: SPAK and WNK kinases: a new target for blood pressure treatment? *Curr Opin Nephrol Hypertens* 2011;20:16-22.
- 49 Huang CL, Yang SS, Lin SH: Mechanism of regulation of renal ion transport by WNK kinases. *Curr Opin Nephrol Hypertens* 2008;17:519-525.
- 50 Mercier-Zuber A, O'Shaughnessy KM: Role of SPAK and OSR1 signalling in the regulation of NaCl cotransporters. *Curr Opin Nephrol Hypertens* 2011;20:534-540.
- 51 Richardson C, Alessi DR: The regulation of salt transport and blood pressure by the WNK-SPAK/OSR1 signalling pathway. *J Cell Sci* 2008;121:3293-3304.
- 52 Gagnon KB, Delpire E: Molecular physiology of SPAK and OSR1: two Ste20-related protein kinases regulating ion transport. *Physiol Rev* 2012;92:1577-1617.
- 53 Falin RA, Miyazaki H, Strange K: C. elegans STK39/SPAK ortholog-mediated inhibition of ClC anion channel activity is regulated by WNK-independent ERK kinase signaling. *Am J Physiol Cell Physiol* 2011;300:C624-635.
- 54 Falin RA, Morrison R, Ham AJ, Strange K: Identification of regulatory phosphorylation sites in a cell volume- and Ste20 kinase-dependent ClC anion channel. *J Gen Physiol* 2009;133:29-42.
- 55 Miyazaki H, Strange K: Differential regulation of a ClC anion channel by SPAK kinase ortholog-mediated multisite phosphorylation. *Am J Physiol Cell Physiol* 2012;302:C1702-1712.
- 56 Park HW, Nam JH, Kim JY, Namkung W, Yoon JS, Lee JS, Kim KS, Venglovecz V, Gray MA, Kim KH, Lee MG: Dynamic regulation of CFTR bicarbonate permeability by [Cl⁻]_i and its role in pancreatic bicarbonate secretion. *Gastroenterology* 2010;139:620-631.
- 57 Alessi DR, Zhang J, Khanna A, Hochdorfer T, Shang Y, Kahle KT: The WNK-SPAK/OSR1 pathway: master regulator of cation-chloride cotransporters. *Sci Signal* 2014;7:re3.
- 58 Kahle KT, Delpire E: Kinase-KCC2 coupling: Cl⁻ rheostasis, disease susceptibility, therapeutic target. *J Neurophysiol* 2015;10.1152/jn.00865.2015jn 00865 02015.
- 59 Watanabe M, Fukuda A: Development and regulation of chloride homeostasis in the central nervous system. *Front Cell Neurosci* 2015;9:371.
- 60 Ureche ON, Baltaev R, Ureche L, Strutz-Seebohm N, Lang F, Seebohm G: Novel insights into the structural basis of pH-sensitivity in inward rectifier K⁺ channels Kir2.3. *Cell Physiol Biochem* 2008;21:347-356.
- 61 Limberg MM, Zumhagen S, Netter MF, Coffey AJ, Grace A, Rogers J, Bockelmann D, Rinne S, Stallmeyer B, Decher N, Schulze-Bahr E: Non dominant-negative KCNJ2 gene mutations leading to Andersen-Tawil syndrome with an isolated cardiac phenotype. *Basic Res Cardiol* 2013;108:353.
- 62 Pathare G, Foller M, Daryadel A, Mutig K, Bogatkov E, Fajol A, Almilaji A, Michael D, Stange G, Voelkl J, Wagner CA, Bachmann S, Lang F: OSR1-Sensitive Renal Tubular Phosphate Reabsorption. *Kidney Blood Press Res* 2012;36:149-161.
- 63 Almilaji A, Munoz C, Hosseinzadeh Z, Lang F: Upregulation of Na⁺,Cl⁻-coupled betaine/gamma-amino-butyric acid transporter BGT1 by Tau tubulin kinase 2. *Cell Physiol Biochem* 2013;32:334-343.
- 64 Almilaji A, Honisch S, Liu G, Elvira B, Ajay SS, Hosseinzadeh Z, Ahmed M, Munoz C, Sopjani M, Lang F: Regulation of the voltage gated K channel Kv1.3 by recombinant human klotho protein. *Kidney Blood Press Res* 2014;39:609-622.
- 65 Hosseinzadeh Z, Dong L, Bhavsar SK, Warsi J, Almilaji A, Lang F: Upregulation of peptide transporters PEPT1 and PEPT2 by Janus kinase JAK2. *Cell Physiol Biochem* 2013;31:673-682.
- 66 Pakladok T, Almilaji A, Munoz C, Alesutan I, Lang F: PIKfyve sensitivity of hERG channels. *Cell Physiol Biochem* 2013;31:785-794.
- 67 Almilaji A, Sopjani M, Elvira B, Borrás J, Dermaku-Sopjani M, Munoz C, Warsi J, Lang UE, Lang F: Upregulation of the creatine transporter Slc6A8 by Klotho. *Kidney Blood Press Res* 2014;39:516-525.
- 68 Fezai M, Elvira B, Borrás J, Ben-Attia M, Hosseinzadeh Z, Lang F: Negative regulation of the creatine transporter SLC6A8 by SPAK and OSR1. *Kidney Blood Press Res* 2014;39:546-554.

- 69 Almilaji A, Szteyn K, Fein E, Pakladok T, Munoz C, Elvira B, Towhid ST, Alesutan I, Shumilina E, Bock CT, Kandolf R, Lang F: Down-regulation of Na/K+ atpase activity by human parvovirus B19 capsid protein VP1. *Cell Physiol Biochem* 2013;31:638-648.
- 70 Hosseinzadeh Z, Luo D, Sopjani M, Bhavsar SK, Lang F: Down-regulation of the epithelial Na(+) channel ENaC by Janus kinase 2. *J Membr Biol* 2014;247:331-338.
- 71 Hosseinzadeh Z, Sopjani M, Pakladok T, Bhavsar SK, Lang F: Downregulation of KCNQ4 by Janus kinase 2. *J Membr Biol* 2013;246:335-341.
- 72 Munoz C, Almilaji A, Setiawan I, Foller M, Lang F: Up-regulation of the inwardly rectifying K(+) channel Kir2.1 (KCNJ2) by protein kinase B (PKB/Akt) and PIKfyve. *J Membr Biol* 2013;246:189-197.
- 73 Warsi J, Dong L, Elvira B, Salker MS, Shumilina E, Hosseinzadeh Z, Lang F: SPAK dependent regulation of peptide transporters PEPT1 and PEPT2. *Kidney Blood Press Res* 2014;39:388-398.
- 74 Warsi J, Elvira B, Bissinger R, Shumilina E, Hosseinzadeh Z, Lang F: Downregulation of peptide transporters PEPT1 and PEPT2 by oxidative stress responsive kinase OSR1. *Kidney Blood Press Res* 2014;39:591-599.
- 75 Warsi J, Hosseinzadeh Z, Elvira B, Bissinger R, Shumilina E, Lang F: Regulation of ClC-2 activity by SPAK and OSR1. *Kidney Blood Press Res* 2014;39:378-387.
- 76 Alesutan IS, Ureche ON, Laufer J, Klaus F, Zurn A, Lindner R, Strutz-Seeböhm N, Tavaré JM, Boehmer C, Palmada M, Lang UE, Seeböhm G, Lang F: Regulation of the glutamate transporter EAAT4 by PIKfyve. *Cell Physiol Biochem* 2010;25:187-194.
- 77 Dermaku-Sopjani M, Almilaji A, Pakladok T, Munoz C, Hosseinzadeh Z, Blecua M, Sopjani M, Lang F: Down-regulation of the Na+-coupled phosphate transporter NaPi-IIa by AMP-activated protein kinase. *Kidney Blood Press Res* 2013;37:547-556.
- 78 Elvira B, Munoz C, Borrás J, Chen H, Warsi J, Ajay SS, Shumilina E, Lang F: SPAK and OSR1 dependent down-regulation of murine renal outer medullary K channel ROMK1. *Kidney Blood Press Res* 2014;39:353-360.
- 79 Lang F, Hoffmann EK: Role of ion transport in control of apoptotic cell death. *Compr Physiol* 2012;2:2037-2061.
- 80 Hoffmann EK: Ion channels involved in cell volume regulation: effects on migration, proliferation, and programmed cell death in non adherent EAT cells and adherent ELA cells. *Cell Physiol Biochem* 2011;28:1061-1078.
- 81 Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. *Physiol Rev* 2009;89:193-277.
- 82 Lang F: Mechanisms and significance of cell volume regulation. *J Am Coll Nutr* 2007;26:613S-623S.
- 83 Liu GX, Derst C, Schlichterl G, Heinen S, Seeböhm G, Bruggemann A, Kummer W, Veh RW, Daut J, Preisig-Müller R: Comparison of cloned Kir2 channels with native inward rectifier K+ channels from guinea-pig cardiomyocytes. *J Physiol* 2001;532:115-126.
- 84 Preisig-Müller R, Schlichterl G, Goerge T, Heinen S, Bruggemann A, Rajan S, Derst C, Veh RW, Daut J: Heteromerization of Kir2.x potassium channels contributes to the phenotype of Andersen's syndrome. *Proc Natl Acad Sci U S A* 2002;99:7774-7779.
- 85 Tristani-Firouzi M, Etheridge SP: Kir 2.1 channelopathies: the Andersen-Tawil syndrome. *Pflugers Arch* 2010;460:289-294.
- 86 Xia M, Jin Q, Bendahhou S, He Y, Larroque MM, Chen Y, Zhou Q, Yang Y, Liu Y, Liu B, Zhu Q, Zhou Y, Lin J, Liang B, Li L, Dong X, Pan Z, Wang R, Wan H, Qiu W, Xu W, Eurlings P, Barhanin J, Chen Y: A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation. *Biochem Biophys Res Commun* 2005;332:1012-1019.
- 87 Howe MW, Feig SL, Osting SM, Haberly LB: Cellular and subcellular localization of Kir2.1 subunits in neurons and glia in piriform cortex with implications for K+ spatial buffering. *J Comp Neurol* 2008;506:877-893.
- 88 Pruss H, Derst C, Lommel R, Veh RW: Differential distribution of individual subunits of strongly inwardly rectifying potassium channels (Kir2 family) in rat brain. *Brain Res Mol Brain Res* 2005;139:63-79.
- 89 Pruss H, Wenzel M, Eulitz D, Thomzig A, Karschin A, Veh RW: Kir2 potassium channels in rat striatum are strategically localized to control basal ganglia function. *Brain Res Mol Brain Res* 2003;110:203-219.
- 90 Stonehouse AH, Pringle JH, Norman RI, Stanfield PR, Conley EC, Brammar WJ: Characterisation of Kir2.0 proteins in the rat cerebellum and hippocampus by polyclonal antibodies. *Histochem Cell Biol* 1999;112:457-465.

- 91 Muessel MJ, Harry GJ, Armstrong DL, Storey NM: SDF-1 α and LPA modulate microglia potassium channels through rho gtpases to regulate cell morphology. *Glia* 2013;61:1620-1628.
- 92 Benfenati V, Caprini M, Nobile M, Rapisarda C, Ferroni S: Guanosine promotes the up-regulation of inward rectifier potassium current mediated by Kir4.1 in cultured rat cortical astrocytes. *J Neurochem* 2006;98:430-445.
- 93 Horio Y: Potassium channels of glial cells: distribution and function. *Jpn J Pharmacol* 2001;87:1-6.
- 94 Jansen LA, Uhlmann EJ, Crino PB, Gutmann DH, Wong M: Epileptogenesis and reduced inward rectifier potassium current in tuberous sclerosis complex-1-deficient astrocytes. *Epilepsia* 2005;46:1871-1880.
- 95 Moussaud S, Lamodièrè E, Savage C, Draheim HJ: Characterisation of K⁺ currents in the C8-B4 microglial cell line and their regulation by microglia activating stimuli. *Cell Physiol Biochem* 2009;24:141-152.
- 96 Obara-Michlewska M, Pannicke T, Karl A, Bringmann A, Reichenbach A, Szeliga M, Hilgier W, Wrzosek A, Szewczyk A, Albrecht J: Down-regulation of Kir4.1 in the cerebral cortex of rats with liver failure and in cultured astrocytes treated with glutamine: Implications for astrocytic dysfunction in hepatic encephalopathy. *J Neurosci Res* 2011;89:2018-2027.
- 97 Ohno Y, Hibino H, Lossin C, Inanobe A, Kurachi Y: Inhibition of astroglial Kir4.1 channels by selective serotonin reuptake inhibitors. *Brain Res* 2007;1178:44-51.
- 98 Pivonkova H, Benesova J, Butenko O, Chvatal A, Anderova M: Impact of global cerebral ischemia on K⁺ channel expression and membrane properties of glial cells in the rat hippocampus. *Neurochem Int* 2010;57:783-794.
- 99 Tsai KL, Chang HF, Wu SN: The inhibition of inwardly rectifying K⁺ channels by memantine in macrophages and microglial cells. *Cell Physiol Biochem* 2013;31:938-951.
- 100 Kang SJ, Cho SH, Park K, Yi J, Yoo SJ, Shin KS: Expression of Kir2.1 channels in astrocytes under pathophysiological conditions. *Mol Cells* 2008;25:124-130.
- 101 Johnston AM, Naselli G, Gonez LJ, Martin RM, Harrison LC, DeAizpurua HJ: SPAK, a STE20/SPS1-related kinase that activates the p38 pathway. *Oncogene* 2000;19:4290-4297.
- 102 Eberhardt C, Amann B, Feuchtinger A, Hauck SM, Deeg CA: Differential expression of inwardly rectifying K⁺ channels and aquaporins 4 and 5 in autoimmune uveitis indicates misbalance in Muller glial cell-dependent ion and water homeostasis. *Glia* 2011;59:697-707.
- 103 Iandiev I, Tenckhoff S, Pannicke T, Biedermann B, Hollborn M, Wiedemann P, Reichenbach A, Bringmann A: Differential regulation of Kir4.1 and Kir2.1 expression in the ischemic rat retina. *Neurosci Lett* 2006;396:97-101.
- 104 Iandiev I, Uckermann O, Pannicke T, Wurm A, Tenckhoff S, Pietsch UC, Reichenbach A, Wiedemann P, Bringmann A, Uhlmann S: Glial cell reactivity in a porcine model of retinal detachment. *Invest Ophthalmol Vis Sci* 2006;47:2161-2171.
- 105 Kofuji P, Biedermann B, Siddharthan V, Raap M, Iandiev I, Milenkovic I, Thomzig A, Veh RW, Bringmann A, Reichenbach A: Kir potassium channel subunit expression in retinal glial cells: implications for spatial potassium buffering. *Glia* 2002;39:292-303.
- 106 Raap M, Biedermann B, Braun P, Milenkovic I, Skatchkov SN, Bringmann A, Reichenbach A: Diversity of Kir channel subunit mRNA expressed by retinal glial cells of the guinea-pig. *Neuroreport* 2002;13:1037-1040.
- 107 Ulbricht E, Pannicke T, Hollborn M, Raap M, Goczalik I, Iandiev I, Hartig W, Uhlmann S, Wiedemann P, Reichenbach A, Bringmann A, Francke M: Proliferative gliosis causes mislocation and inactivation of inwardly rectifying K⁽⁺⁾ (Kir) channels in rabbit retinal glial cells. *Exp Eye Res* 2008;86:305-313.
- 108 Longden TA, Dabertrand F, Hill-Eubanks DC, Hammack SE, Nelson MT: Stress-induced glucocorticoid signaling remodels neurovascular coupling through impairment of cerebrovascular inwardly rectifying K⁺ channel function. *Proc Natl Acad Sci U S A* 2014;111:7462-7467.