Acta Pharm. **71** (2021) 317–324
https://doi.org/10.2478/acph-2021-0017

Short communication

Inhibitory effect of terfenadine on Kir2.1 and Kir2.3 channels

MAYRA DELGADO-RAMÍREZ[1]
FANNY JUNUE RODRIGUEZ-LEAL[1]
ALDO AZMAR RODRÍGUEZ-MENCHACA[2]
ELOY GERARDO MORENO-GALINDO[1]
JOSÉ ANTONIO SANCHEZ-CHAPULA[1]
TANIA FERRER[1,*]

[1] *Centro Universitario de Investigaciones Biomédicas, Universidad de Colima Colima, COL 28045, México*

[2] *Departamento de Fisiología y Biofísica Facultad de Medicina, Universidad Autónoma de San Luis Potosí, San Luis Potosí, SLP 78210, México*

Accepted June 28, 2020
Published online July 9, 2020

Terfenadine is a second-generation H1-antihistamine that despite potentially can produce severe side effects it has recently gained attention due to its anticancer properties. Lately, the subfamily 2 of inward rectifier potassium channels (Kir2) has been implicated in the progression of some tumoral processes. Hence, we characterized the effects of terfenadine on Kir2.x channels expressed in HEK-293 cells. Terfenadine inhibited Kir2.3 channels with a strikingly greater potency (IC_{50} = 1.06 ± 0.11 μmol L^{-1}) compared to Kir2.1 channels (IC_{50} = 27.8 ± 4.8 μmol L^{-1}). The Kir2.3(I213L) mutant, possessing a larger affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2) than the wild-type Kir2.3, was less sensitive to terfenadine inhibition (IC_{50} = 13.0 ± 2.9 μmol L^{-1}). Additionally, the PIP_2 intracellular application had largely reduced the inhibition of Kir2.1 channels by terfenadine. Our data support that Kir2.x channels are targets of terfenadine by affecting their interaction with PIP_2, which could be regarded as a mechanism of the antitumor properties of terfenadine.

Keywords: terfenadine, inward rectifier potassium channels, phosphatidylinositol 4,5-bisphosphate, cationic amphiphilic drugs

Terfenadine is a non-sedating second-generation H1-antihistamine formerly prescribed to treat allergic rhinitis and urticarial (1). This drug was withdrawn from the market (2) due to the risk of fatal arrhythmias (torsades de pointes and ventricular fibrillation) (3) by inhibiting several cardiac ion channels, including hERG (4), $Na_V1.5$ (5), hKv1.5 (6), and Kir3.1 (I_{KACh}) (7).

Terfenadine has recently attracted attention due to important anticancer properties in different experimental models (*in vitro* and *in vivo).* This drug suppresses the spontaneous growth of neoplastic mast cells by an apoptotic mechanism (8). In addition, terfenadine triggers apoptosis in melanoma cells (9) and human hormone unresponsive prostate cancer (10). Notably, terfenadine reduces the tumor growth in breast cancer cells, including both basal cells and cells resistant to trastuzumab (11), and in resistant non-small cell lung cancer when combined with epirubicin (12).

* Correspondence; e-mail: tania@ucol.mx

The Kir2 channels subfamily is composed of four members (Kir2.1- Kir2.4) underlying an inwardly rectifying K^+ current (13, 14). Kir2.x channels are very important for setting and controlling the resting membrane potential of different types of cells (13). Also, these channels help to determine the action potential waveform and the excitability of cardiac myocytes and neurons (13). Interestingly, it has been shown recently that Kir2.x channels are implied in some tumoral processes. To mention a few, the expression of Kir2.1 is increased in stomach cancer cells controlling invasion and metastasis (15) and is associated with drug resistance of small-cell lung cancer (16). Kir2.2 is involved in the *in vivo* progression of tumors derived from PC3 cells (a human prostate adenocarcinoma) (17). In turn, Kir2.3 has been implicated in the growth and metastasis of lung adenocarcinoma (18).

As reported by previous studies, Kir channels are inhibited by several drugs with a distinctive structure, the so-called cationic amphiphilic drugs (CADs) (19). These compounds are characterized by their archetypical amphiphilic structure determined by a hydrophobic region (aliphatic or aromatic rings) and a hydrophilic moiety that includes an amino group charged at physiological pH (19). Therefore, given the terfenadine's CAD-like type of nature (Fig. 1a) and its anti-tumoral properties, as well as the important role of Kir2.x channels in carcinogenesis, we have investigated the hypothesis that terfenadine inhibits Kir2.x channels and determined the underlying mechanism of action in this study.

EXPERIMENTAL

Drug and reagents

Terfenadine (purity > 97.5 %) was purchased from Sigma-Aldrich (USA) and dissolved in DMSO to prepare a 10 mmol L^{-1} stock solution, which was diluted in the extracellular (bath) solution to final concentrations as required. L-α phosphatidylinositol 4,5-bisphosphate (PIP_2, (Avanti Polar Lipids, USA) was aliquoted, dried, and stored at –70 °C. Aliquots were diluted to 10 μmol L^{-1} in the intracellular (pipette) solution and sonicated on ice for 15 min before application.

Cell culture and cDNA expression

We carried out the experiments in HEK-293 cells that were cultured under standard conditions in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10 % fetal bovine serum (Gibco) and 1 % antibiotic-antimycotic solution (Sigma-Aldrich) at 37 °C in a humidified incubator (5 % CO_2). The Lipofectamine 2000 reagent (Invitrogen, USA) was used for transiently transfecting HEK-293 cells with human cDNAs (2 μg) encoding Kir2.1, Kir2.3 (provided by C. Vandenberg from the University of California, Santa Barbara, CA, USA), or Kir2.3(I213L) channels 24 h before the experiments. The QuickChange Site-Directed Mutagenesis kit (Stratagene, USA) was employed to prepare the Kir2.3(I213L) mutant channel. DNA sequencing was used to validate the mutation. Transfected cells were identified using the green fluorescent protein (GFP), which was co-transfected with the Kir2.x cDNAs.

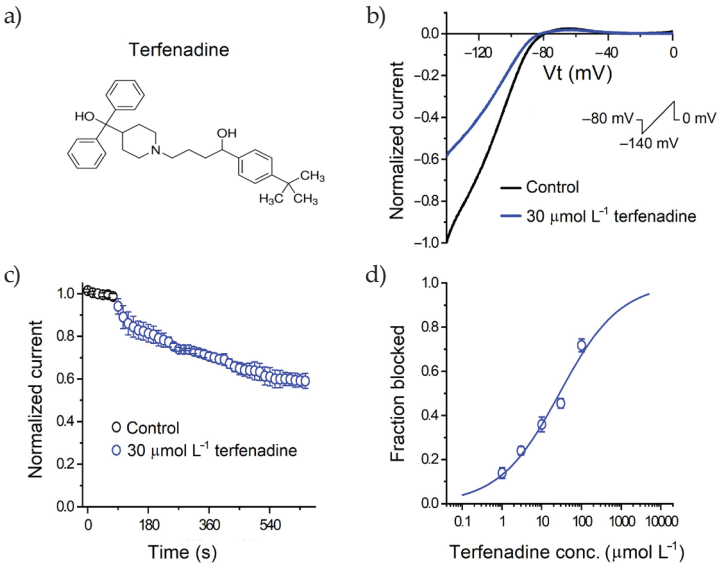

Fig. 1. Decrease of Kir2.1 currents produced by terfenadine. a) Chemical structure of terfenadine. b) Representative Kir2.1 current traces before and after 30 μmol L^{-1} terfenadine application. In all figures containing current traces, the inset shows the voltage protocol used to elicit the currents. Currents were normalized to that obtained at –140 mV in control conditions (the holding current is not shown). c) Mean data of the normalized current recorded at –140 mV in control conditions and during perfusion of 30 μmol L^{-1} terfenadine, plotted as a function of the perfusion time. d) The concentration-effect relationship for inhibition of Kir2.1 current at –140 mV by terfenadine. Values of IC_{50} and Hill slope (H) are mentioned in the text; $n = 5$.

Electrophysiological recordings

Macroscopic current recordings were performed at room temperature (22–24 °C) using the patch-clamp method in the whole-cell configuration. We employed the pCLAMP 9 software (Molecular Devices, USA), an Axopatch 200B amplifier (Molecular Devices) and a Digidata 1440A interface (Molecular Devices) to acquire data and generate pulses. Currents were low-pass filtered at 1 kHz and digitized at 5 kHz. Patch electrodes were fabricated from borosilicate glass (World Precision Instruments, USA) in a programmable puller (Sutter Instruments, USA). Micropipettes had tip resistances between 1.5 and 2.5 MΩ after being filled with the intracellular (pipette) solution. The extracellular (bath) solution without (control) or with terfenadine was applied using a rapid switching device (VC-77SP Warner Instruments, USA), and recordings were obtained when reaching steady--state conditions. The intracellular (pipette) solution composition was (in mmol L^{-1}): KCl, 110; HEPES, 10; K_4BAPTA, 5; K_2ATP, 5; and $MgCl_2$, 1 (pH was adjusted to 7.2 with KOH). The extracellular (bath) solution had the following composition (in mmol L^{-1}): NaCl, 130; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 10; and glucose, 10 (pH was adjusted to 7.4 with NaOH). The bath was grounded through an agar-KCl bridge. All current traces are shown as the currents sensitive to 2 mmol L^{-1} $BaCl_2$.

Data analysis

For analyzing current recordings, we used the software pClamp 10.6 (Molecular Devices) and Origin 8 (OriginLab Corp., USA). Concentration-response curves were fitted with a Hill equation ($f = 1/\{1+ (IC_{50})/[\text{terfenadine}]^{H}\}$; where f is the fractional block of the current and H the Hill coefficient. Statistical analysis (Origin 8; OriginLab Corp.) was performed using the unpaired Student's t-test after evaluating the normal distribution of data with the Shapiro-Wilk test. Results are expressed as mean ± SEM, and the significance of the differences was assumed at $p < 0.05$ (two-tailed).

RESULTS AND DISCUSSION

Terfenadine inhibits the Kir2.1 and Kir2.3 channels

In this study, we examined whether terfenadine affects Kir2.1 and Kir2.3 channels and the underlying mechanism of action. To this end, HEK-293 cells were used to express Kir2.x channels because they are a good model to study the biophysical properties of exogenous ion channels in isolation; they are easily transfected and voltage-clamped, and their small endogenous currents do not interfere with the analysis of the studied current (20). Thus, Kir2.1 and Kir2.3 currents were evaluated using a voltage-ramp protocol (3 s duration) from –140 to 0 mV every 15 s and a holding potential of –80 mV. Fig. 1b shows representative Kir2.1 current traces in control conditions and after the perfusion of 30 μmol L^{-1} terfenadine. The development of the terfenadine effect on Kir2.1 currents was slow as depicted in the time course of inhibition (Fig. 1c). Terfenadine inhibition on Kir2.1 currents increased when augmenting the drug concentration: the concentration-response relationship (measured at –140 mV) yielded an IC_{50} of 27.8 ± 4.8 μmol L^{-1} and H of 0.56 ± 0.06 (Fig. 1d).

In turn, the effect of terfenadine on Kir2.3 currents was strikingly more potent than that on Kir2.1, since ~ 50 % of the current was inhibited by 1 μmol L^{-1} terfenadine (Fig. 2a); although the time course was similarly slow (Fig. 2b). The IC_{50} to inhibit Kir2.3 channels

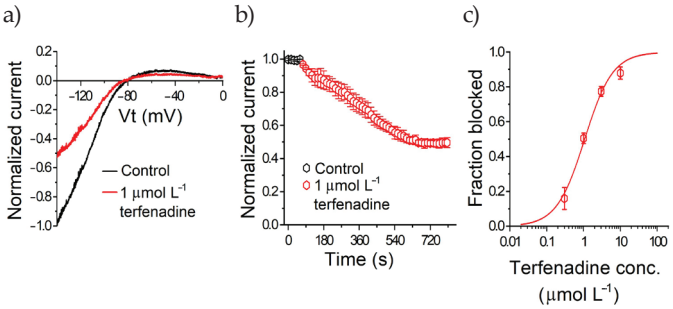

Fig. 2. Terfenadine inhibits Kir2.3 channels. a) Illustrative recordings of Kir2.3 currents evoked in control conditions and after perfusion of 1 μmol L^{-1} terfenadine. b) Time course of development of Kir2.3 current inhibition by 1 μmol L^{-1} terfenadine at –140 mV. c) A concentration-response curve of inhibited Kir2.3 current at –140 mV; $n = 5$.

was 1.06 ± 0.11 μmol L^{-1} (*H* of 1.14 ± 0.14) (Fig. 2c), which is ~ 26-fold lower in comparison to that for Kir2.1. The slow time courses of inhibition and the different potency of terfenadine to inhibit Kir2.1 and Kir2.3 channels resemble those of CADs, whose mechanism of action is to interfere with the Kir channel-PIP_2 interaction (21). The effect of terfenadine on Kir2.1 and Kir2.3 channels was inversely correlated with the apparent affinity of these channels for PIP_2 (20), the lower the affinity for PIP_2, the greater the potency of terfenadine to inhibit the channel. Thus, we next proceeded to test this hypothesis considering the CAD-type nature of terfenadine.

Kir2.3(I213L) mutant channel is less sensitive to terfenadine inhibition

Compared to the wild-type Kir2.3, Kir2.3(I213L) mutant channel has a higher apparent affinity for PIP_2, and thus, it is less sensitive to inhibitory drugs (21). Hence, we assessed the effect of terfenadine on this mutant channel to investigate the inhibitory mechanism of this compound. Terfenadine decreased Kir2.3(I213L) currents with lesser potency (10 μmol L^{-1} terfenadine diminished the current in ~ 50 %) (Fig. 3a) than that for wild-type Kir2.3. The time course of the terfenadine effect on Kir2.3(I213L) was also slow (Fig. 3b). An IC_{50} of 13.0 ± 2.9 μmol L^{-1} (*H* of 0.77 ± 0.16) was obtained from the concentration-response relationship (Fig. 3c), resulting ~12-fold greater than that observed for wild-type Kir2.3 channels. These data suggest that the interaction between PIP_2 and Kir2.x channels is affected by terfenadine.

Terfenadine is a basic compound with a moderate degree of lipophilicity (log *D* = 2.11, pK_a = 8.6) that could interact with the hydrophobic and hydrophilic moieties of phospholipids, particularly with those negatively charged (22). Other drugs with the same characteristics inhibit Kir channels by several mechanisms, but it has been shown that such drugs mainly affect the interaction of the channels with PIP_2 (19). In this regard, it was recently reported that terfenadine inhibits Kir3.1 channels by binding to a region below the residue F137 (a pore-helix amino acid) and amid the transmembrane helices from two contiguous subunits. Binding of terfenadine to this region sterically interferes with the PIP_2-channel interaction, suggesting that this could be the mechanism of inhibition (7).

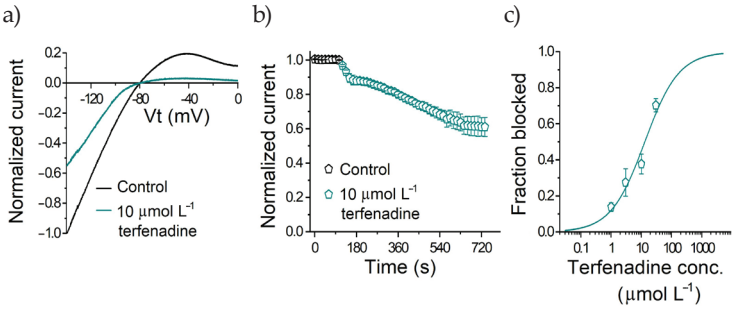

Fig. 3. Effects of terfenadine on the mutated channel Kir2.3(I213L). a) Normalized recordings of Kir2.3(I213L) current obtained previously and following exposure to10 μM terfenadine. b) Time course of inhibition (at –140 mV) of Kir2.3(I213L) channels by 10 μmol L^{-1} terfenadine. c) Terfenadine concentration plotted as a function of the Kir2.3(I213L) current inhibition at –140 mV; *n* = 5.

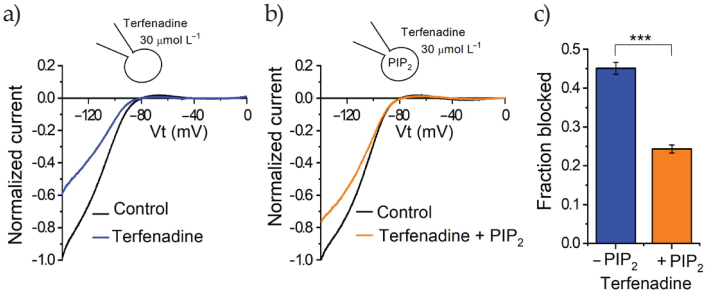

Fig. 4. Effect of terfenadine on Kir2.1 currents in the presence of exogenous PIP_2. Representative Kir2.1 current traces obtained before and after application of 30 μmol L^{-1} terfenadine: a) without and b) with 10 μmol L^{-1} of PIP_2 dialyzed in the pipette solution. c) Fractional block of Kir2.1 channels by terfenadine under control conditions (–PIP_2) and in the presence (+ PIP_2) of exogenous PIP_2 in the patch pipette (n = 5 and 6 for –PIP_2 and + PIP_2 groups, respectively), ***$p < 0.001$.

Exogenous PIP_2 attenuates the inhibition of Kir2.1 channels by terfenadine

To support the hypothesis that terfenadine's mechanism of action could be by interfering with the Kir2.x channel-PIP_2 interaction, we performed an additional experiment by dialyzing (for 5 min in the patch pipette) exogenous PIP_2 before the terfenadine application in the extracellular (bath) solution in Kir2.1 expressing cells. Fig. 4a,b depicts the effect of 30 μmol L^{-1} terfenadine on Kir2.1 currents in control conditions and after intracellular perfusion of 10 μmol L^{-1} PIP_2. When PIP_2 was present in the patch pipette, terfenadine inhibition was reduced ~ 50 %, since the percentage inhibition at –140 mV was 24.3 ± 1.0 % (n = 5), compared to 45.1 ± 1.5 % (n = 6) in cells recorded in the absence of PIP_2 (Fig. 4c).

Overall, our data suggest that terfenadine targets the PIP_2-channel interaction to inhibit the channels. First, the drug inhibited Kir2.x channels with a slow time course, which is characteristic of this mechanism of action (19), where the drug probably inserts into the lipid membrane. Second, strengthening the interaction of the channel and PIP_2 (by using the Kir2.3(I213L) mutant channel) decreased the terfenadine inhibition. Third, supplying exogenous PIP_2 to the intracellular milieu reduced the potency of terfenadine to inhibit Kir2.1 channels. Taken together, our results support that Kir2.1 and Kir2.3 channels are inhibited by terfenadine due to the interference of the channel interaction with PIP_2.

Given the role of K^+ channels on tumoral processes, in recent years their pharmacological inhibition has been considered as a promising strategy against carcinogenesis, either by reducing the proliferation and/or decreasing the invasiveness and cell migration in different cancer cell types (23–25). Therefore, this work supports the potential application of terfenadine derivatives with fewer side effects as a part of anticancer therapy in Kir2.x expressing malignant cells, although, further studies (*in vitro* and *in vivo*) are needed to elucidate the effect of terfenadine on the malignant proliferation and metastasis of these types of tumoral cells.

CONCLUSIONS

Terfenadine was more potent to inhibit Kir2.3 than Kir2.1 channels, and the mutation Kir2.3(I213L) decreased the terfenadine effect compared to that on Kir2.3, *i.e.* the stronger

the affinity of the channel for PIP_2, the lower the potency of terfenadine to inhibit this channel. We also found that the PIP_2 intracellular application decreased the potency of terfenadine to inhibit Kir2.1 channels. Our results suggest that terfenadine interferes with the PIP_2-channel interaction. This could be an alternative mechanism contributing to the anticancer properties of terfenadine and could have a potential application in tumors where Kir2 channels have a relevant role in proliferation and metastasis.

Acknowledgments. – We are grateful to QFB Miguel Ángel Flores Virgen (Centro Universitario de Investigaciones Biomédicas de la Universidad de Colima, Colima, Col. Mexico) for the technical assistance. M. D-R. is a Postdoctoral Fellow (# 740437) of the Consejo Nacional de Ciencia y Tecnología (Conacyt, Mexico). This work was supported by SEP-Conacyt grants: CB-2010-01-153394 (to T.F.) and CB-2013-01-220546 (to J.A.S.-C.).

REFERENCES

1. D. McTavish, K. L. Goa and M. Ferrill, Terfenadine. An updated review of its pharmacological properties and therapeutic efficacy, *Drugs* **39** (1990) 552–574; https://doi.org/10.2165/00003495-199039040-00006
2. F. Register, Proposal to withdraw approval of two new drug applications and one abbreviated new drug application; opportunity for a hearing. Food and Drug Administration [Docket No. 96N-0512], *Federal Register* **62** (1997) 2–9.
3. T. J. MacConnell and A. J. Stanners, Torsades de pointes complicating treatment with terfenadine, *BMJ* **302** (1991) 1469; https://doi.org/10.1136/bmj.302.6790.1469-b
4. K. Kamiya, R. Niwa, M. Morishima, H. Honjo and M. C. Sanguinetti, Molecular determinants of hERG channel block by terfenadine and cisapride, *J. Pharmacol. Sci*. **108** (2008) 301–307; https://doi.org/10.1254/jphs.08102fp
5. H. R. Lu, A. N. Hermans and D. J. Gallacher, Does terfenadine-induced ventricular tachycardia/fibrillation directly relate to its QT prolongation and Torsades de Pointes?, *Br. J. Pharmacol*. **166** (2012) 1490–1502; https://doi.org/10.1111/j.1476-5381.2012.01880.x
6. C. Valenzuela, E. Delpon, L. Franqueza, P. Gay, J. Vicente and J. Tamargo, Comparative effects of nonsedating histamine H1 receptor antagonists, ebastine and terfenadine, on human Kv1.5 channels, *Eur. J. Pharmacol*. **326** (1997) 257–263; https://doi.org/10.1016/s0014-2999(97)85421-0
7. I. S. Chen, C. Liu, M. Tateyama, I. Karbat, M. Uesugi, E. Reuveny and Y. Kubo, Non-sedating antihistamines block G-protein-gated inwardly rectifying K(+) channels, *Br. J. Pharmacol*. **176** (2019) 3161–3179; https://doi.org/10.1111/bph.14717
8. E. Hadzijusufovic, B. Peter, K. V. Gleixner, K. Schuch, W. F. Pickl, T. Thaiwong, V. Yuzbasiyan-Gurkan, I. Mirkina, M. Willmann and P. Valent, H1-receptor antagonists terfenadine and loratadine inhibit spontaneous growth of neoplastic mast cells, *Exp. Hematol*. **38** (2010) 896–907; https://doi.org/10.1016/j.exphem.2010.05.008
9. F. Nicolau-Galmes, A. Asumendi, E. Alonso-Tejerina, G. Perez-Yarza, S. M. Jangi, J. Gardeazabal, Y. Arroyo-Berdugo, J. M. Careaga, J. L. Diaz-Ramon, A. Apraiz and M. D. Boyano, Terfenadine induces apoptosis and autophagy in melanoma cells through ROS-dependent and -independent mechanisms, *Apoptosis* **16** (2011) 1253–1267; https://doi.org/10.1007/s10495-011-0640-y
10. W. T. Wang, Y. H. Chen, J. L. Hsu, W. J. Leu, C. C. Yu, S. H. Chan, Y. F. Ho, L. C. Hsu and J. H. Guh, Terfenadine induces anti-proliferative and apoptotic activities in human hormone-refractory prostate cancer through histamine receptor-independent Mcl-1 cleavage and Bak up-regulation, *Naunyn Schmiedebergs Arch. Pharmacol*. **387** (2014) 33–45; https://doi.org/10.1007/s00210-013-0912-x

11. P. Fernandez-Nogueira, A. Noguera-Castells, G. Fuster, L. Recalde-Percaz, N. Moragas, A. Lopez-Plana, E. Enreig, P. Jauregui, N. Carbo, V. Almendro, P. Gascon, P. Bragado and M. Mancino, Histamine receptor 1 inhibition enhances antitumor therapeutic responses through extracellular signal-regulated kinase (ERK) activation in breast cancer, *Cancer Lett.* **424** (2018) 70–83; https://doi.org/10.1016/j.canlet.2018.03.014
12. L. An, D. D. Li, H. X. Chu, Q. Zhang, C. L. Wang, Y. H. Fan, Q. Song, H. D. Ma, F. Feng and Q. C. Zhao, Terfenadine combined with epirubicin impedes the chemo-resistant human non-small cell lung cancer both in vitro and in vivo through EMT and Notch reversal, *Pharmacol. Res.* **124** (2017) 105–115; https://doi.org/10.1016/j.phrs.2017.07.021
13. H. Hibino, A. Inanobe, K. Furutani, S. Murakami, I. Findlay and Y. Kurachi, Inwardly rectifying potassium channels: their structure, function, and physiological roles, *Physiol. Rev.* **90** (2010) 291–366; https://doi.org/10.1152/physrev.00021.2009
14. P. Sulaiman, Y. Xu, M. E. Fina, S. R. Tummala, H. Ramakrishnan, A. Dhingra and N. Vardi, Kir2.4 surface expression and basal current are affected by heterotrimeric G-proteins, *J. Biol. Chem.* **288** (2013) 7420–7429; https://doi.org/10.1074/jbc.M112.412791
15. C. D. Ji, Y. X. Wang, D. F. Xiang, Q. Liu, Z. H. Zhou, F. Qian, L. Yang, Y. Ren, W. Cui, S. L. Xu, X. L. Zhao, X. Zhang, Y. Wang, P. Zhang, J. M. Wang, Y. H. Cui and X. W. Bian, Kir2.1 Interaction with Stk38 promotes invasion and metastasis of human gastric cancer by enhancing MEKK2-MEK1/2-ERK1/2 signaling, *Cancer Res.* **78** (2018) 3041–3053; https://doi.org/10.1158/0008-5472.CAN-17-3776
16. H. Liu, J. Huang, J. Peng, X. Wu, Y. Zhang, W. Zhu and L. Guo, Upregulation of the inwardly rectifying potassium channel Kir2.1 (KCNJ2) modulates multidrug resistance of small-cell lung cancer under the regulation of miR-7 and the Ras/MAPK pathway, *Mol. Cancer* **14** (2015) 59; https://doi.org/10.1186/s12943-015-0298-0
17. I. Lee, S. J. Lee, T. M. Kang, W. K. Kang and C. Park, Unconventional role of the inwardly rectifying potassium channel Kir2.2 as a constitutive activator of RelA in cancer, *Cancer Res.* **73** (2013) 1056–1062; https://doi.org/10.1158/0008-5472.CAN-12-2498
18. X. Y. Wu and X. Y. Yu, Overexpression of KCNJ4 correlates with cancer progression and unfavorable prognosis in lung adenocarcinoma, *J. Biochem. Mol. Toxicol.* **33** (2019) e22270; https://doi.org/10.1002/jbt.22270
19. M. A. van der Heyden, A. Stary-Weinzinger and J. A. Sanchez-Chapula, Inhibition of cardiac inward rectifier currents by cationic amphiphilic drugs, *Curr. Mol. Med.* **13** (2013) 1284–1298; https://doi.org/10.2174/15665240113139990043
20. P. Thomas and T. G. Smart, HEK293 cell line: a vehicle for the expression of recombinant proteins, *J. Pharmacol. Toxicol. Methods.* **51** (2005) 187–200; https://doi.org/10.1016/j.vascn.2004.08.014
21. X. Du, H. Zhang, C. Lopes, T. Mirshahi, T. Rohacs and D. E. Logothetis, Characteristic interactions with phosphatidylinositol 4,5-bisphosphate determine regulation of kir channels by diverse modulators, *J. Biol. Chem.* **279** (2004) 37271–37281; https://doi.org/10.1074/jbc.M403413200
22. S. Przestalski, J. Sarapuk, H. Kleszczynska, J. Gabrielska, J. Hladyszowski, Z. Trela and J. Kuczera, Influence of amphiphilic compounds on membranes, *Acta Biochim. Pol.* **47** (2000) 627–638.
23. J. J. Babcock and M. Li, hERG channel function: beyond long QT, *Acta Pharmacol. Sin.* **34** (2013) 329–335; https://doi.org/10.1038/aps.2013.6
24. G. D'Alessandro, M. Catalano, M. Sciaccaluga, G. Chece, R. Cipriani, M. Rosito, A. Grimaldi, C. Lauro, G. Cantore, A. Santoro, B. Fioretti, F. Franciolini, H. Wulff and C. Limatola, KCa3.1 channels are involved in the infiltrative behavior of glioblastoma in vivo, *Cell Death Dis.* **4** (2013) e773; https://doi.org/10.1038/cddis.2013.279
25. M. Nunez, V. Medina, G. Cricco, M. Croci, C. Cocca, E. Rivera, R. Bergoc and G. Martin, Glibenclamide inhibits cell growth by inducing G0/G1 arrest in the human breast cancer cell line MDA-MB-231, *BMC Pharmacol. Toxicol.* **14** (2013) 6; https://doi.org/10.1186/2050-6511-14-6