

- sodium channels in K562 cells. *Cell. Tiss. Biol. (Tsitologiya)* 60 (10) : 821–825.
- Филатова Н.А., Чубинский-Надеждин В.И., Иванов В.А., Морачевская Е.А. 2010. Чувствительность к действию естественных киллеров зависит от целостности липидных рафтов в мембране трансформированных клеток. *Цитология*. 52 (12) : 983–989. (Filatova N.A., Chubinskiy-Nadezhdin V.I., Ivanov V.A., Morachevskaya E.A. 2010. Sensitivity to lysis by natural killers depends on the integrity of lipid rafts in plasma membrane of transformed cells. *Tsitologiya*. 52 (12) : 983–989.)
- Чубинский-Надеждин В.И., Ефремова Т.Н., Негуляев Ю.А., Морачевская Е.А. 2018. Сопряженная активация механочувствительных и кальцийзависимых калиевых каналов в клетках 3Т3 и 3Т3-SV40. *Цитология*. 60 (1) : 14–20. (Chubinskiy-Nadezhdin V.I., Efremova T.N., Negulyaev Y.A., Morachevskaya E.A. 2018. Coupled activation of mechanosensitive and calcium-dependent potassium channels in 3T3 and 3T3-SV40 cells. *Cell Tiss. Biol.* 12 (3) : 231–237.)
- Arnadóttir J., Chalfie M. 2010. Eukaryotic mechanosensitive channels. *Annu. Rev. Biophys.* 39 : 111–137.
- Chubinskiy-Nadezhdin V. I., Negulyaev Y. A., Morachevskaya E. A. 2011. Cholesterol depletion-induced inhibition of stretch-activated channels is mediated via actin rearrangement. *Biochem. Biophys. Res. Commun.* 412 : 80–85.
- Chubinskiy-Nadezhdin V.I., Negulyaev Y.A., Morachevskaya E.A. 2014. Functional coupling of ion channels in cellular mechanotransduction. *Biochem. Biophys. Res. Commun.* 451 : 421–424.
- Chubinskiy-Nadezhdin V.I., Vasileva V.Y., Pugovkina N.A., Vassilieva I.O., Morachevskaya E.A., Nikolsky N.N., Negulyaev Y.A. 2017. Local calcium signalling is mediated by mechanosensitive ion channels in mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* 482 : 563–568.
- Chubinskiy-Nadezhdin V.I., Vasileva V.Y., Vassilieva I.O., Sudarikova A.V., Morachevskaya E.A., Negulyaev Y.A. 2019. Agonist-induced Piezo1 activation suppresses migration of transformed fibroblasts. *Biochem. Biophys. Res. Commun.* 514: 173–179.
- Coste B., Mathur J., Schmidt M., Earley T.J., Ranade S., Petrus M.J., Dubin A.E., Patapoutian A. 2010. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science*. 330 : 55–60.
- Guéguinou M., Chantôme A., Fromont G., Bougnoux P., Vandier C., Potier-Cartereau M. 2014. K_{Ca} and Ca^{2+} channels: The complex thought. *Biochim. Biophys. Acta.* 1843 2322–2333.
- Hamill O.P., McBride D.W. 1996. The pharmacology of mechanogated membrane ion channels. *Pharmacol. Rev.* 48 : 231–252.
- Leary J.F., Ohlsson-Wilhelm B.M., Giuliano R., LaBella S., Farley B., Rowley P.T. 1987. Multipotent human hematopoietic cell line K562: lineage-specific constitutive and inducible antigens. *Leuk. Res.* 11 : 807–815.
- Maroto R., Hamill O.P. 2007. MscCa regulation of tumor cell migration and metastasis. In: *Mechanosensitive channels. (Part B). Current Topics in Membranes.* Amsterdam: Elsevier. 59 : 485–509.
- Maroto R., Kurosky A., Hamill O.P. 2012. Mechanosensitive Ca^{2+} permeant cation channels in human prostate tumor cells. *Channels (Austin)*. 6 : 290–307.
- McHugh B., Murdoch A., Haslett C., Sethi T. 2012. Loss of the integrin-activating transmembrane protein Fam38A (Piezo1) promotes a switch to a reduced integrin-dependent mode of cell migration. *PLoS One*. 7 : e40346.
- Morachevskaya E., Sudarikova A., Negulyaev Y. 2007. Mechanosensitive channel activity and F-actin organization in cholesterol-depleted human leukaemia cells. *Cell Biol. Int.* 31 : 374–381.
- Negulyaev Y.A., Khaitlina S.Y., Hinssen H., Shumilina E.V., Vedernikova E.A. 2000. Sodium channel activity in leukemia cells is directly controlled by actin polymerization, *J. Biol. Chem.* 275 : 40933–40937.
- Negulyaev Y.A., Vedernikova E.A., Kinev A.V., Voronin A.P. 1996. Exogenous heat shock protein hsp70 activates potassium channels in U937 cells. *Biochim. Biophys. Acta.* 1282 : 156–162.
- Simons K., Toomre D. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* 1 : 31–39.
- Statuschenko A.V., Negulyaev Y.A., Morachevskaya E.A. 2005. Actin cytoskeleton disassembly affects conductive properties of stretch-activated cation channels in leukaemia cells. *Biochim. Biophys. Acta.* 1669 : 53–60.
- Statuschenko A.V., Sudarikova A.V., Negulyaev Y.A., Morachevskaya E.A. 2006. Magnesium permeation through mechanosensitive channels: single-current measurements. *Cell Res.* 16 : 723–730.
- Statuschenko A.V., Vedernikova E.A. 2002. Mechanosensitive cation channels in human leukaemia cells: calcium permeation and blocking effect. *J. Physiol.* 541 : 81–90.

FUNCTIONAL COUPLING OF ION CHANNELS IN THE PROCESS OF MECHANO-DEPENDENT ACTIVATION IN K562 CELLS

V. I. Chubinskiy-Nadezhdin^{a, *}, Yu. A. Negulyaev^{a, b}, and E. A. Morachevskaya^a

^aInstitute of Cytology RAS, St. Petersburg 194064 Russia

^bPeter the Great St. Petersburg Polytechnic University, St. Petersburg, 195251 Russia

*e-mail: vchubinskiy@gmail.com

Mechanically gated cation channels that are activated by plasma membrane deformation are the key players in the transduction of mechanical signals from cell surface to cytoplasmic structures. It remained unclear how mechano-dependent reactions involving ion channels are realized in native cells. In this study, we analyzed the development of single channel activity in human myeloid leukemia K562 cell line in response to the application of mechanical stimulus by stretching the fragment of plasma membrane. Registration of ionic currents using the classical variants

of patch clamp method revealed functional clustering and the interaction of various types of the channels in the plasma membrane during mechanotransduction. Particularly, coupled activation of mechanosensitive calcium-permeable channels and potassium calcium-activated channels was found in K562 cells. Real-time current records demonstrate that calcium influx from the extracellular environment into the cytoplasm via mechanosensitive channels activates colocalized potassium channels that do not have their own mechanical sensitivity. In experiments on K562 cells and transformed 3T3-SV40 fibroblasts, functional coupling of the channels during their mechano-dependent activation was shown after incubation of cells with F-actin destructor cytochalasin D. The results allow us to assume that functional clusters of potassium SK channels and stretch-activated cation Piezo1/2 channels are presented in plasma membrane of K562 and 3T3-SV40 cells.

Keywords: patch clamp, mechanosensitive channels, cell membrane, local calcium influx, actin cytoskeleton, human myeloid leukemia K562 cells