СОБЛЮДЕНИЕ ЭТИЧЕСКИХ СТАНДАРТОВ

В экспериментах животные и люди не участвовали.

КОНФЛИКТ ИНТЕРЕСОВ

Конфликт интересов отсутствует.

СПИСОК ЛИТЕРАТУРЫ

Москалева Е.Ю., Родина А.В., Семочкина Ю.П., Высоцкая О.В. 2022. Анализ маркеров окислительного повреждения нейронов и нейровоспаления в отдаленный период после γ-облучения головы мышей в разных дозах Радиационная биология. Радиоэкология. Т. 62 № 2. С. 171. (*Moskaleva E.Yu., Rodina A.V., Semochkina Ju.P., Vysotskaya O.V.* 2022. Analysis of neurons damage and level of neuroinflammation late after γ-irradiation of mice head at different doses. Radiacionnaja biologija. Radioekologija. V. 62 № 2. P. 171.)
https://doi.org/10.31857/S0869803122020059

Новиков В.Е., Левченкова О.С., Пожилова Е.В. 2014. Роль активных форм кислорода в физиологии и патологии клетки и их фармакологическая регуляция. Обзоры по клинической фармакологии и лекарственной терапии. Т. 12. № 4. С. 13. (*Novikov V.E., Levchenkova O.S., Pozhilova Y.V.* 2014. Role of reactive oxygen species in cell physiology and pathology and their pharmacological regulation. Rev. Clin. Pharmacol. Drug Ther. Vol. 12. № 4. P. 13.)
https://doi.org/10.17816/RCF12413-21

Askew K., Li K., Olmos-Alonso A., Garcia-Moreno F., Liang Y., Richardson P., Tipton T., Chapman M.A., Riecken K., Beccari S., Sierra A., Molnár Z., Cragg M.S., Garaschuk O., Perry V.H., Gomez-Nicola D. 2017. Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Rep. V. 18. P. 391.
https://doi.org/10.1016/j.celrep.2016.12.041

Bachiller S., Jiménez-Ferrer I., Paulus A., Yang Y., Swanberg M., Deierborg T., Boza-Serrano A. 2018. Microglia in neurological diseases: a road map to brain-disease dependent-inflammatory response. Front. Cell Neurosci. V. 12. P. 488.
https://doi.org/10.3389/fncel.2018.00488

Becher B., Antel J.P. 1996. Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. Glia. V. 18. P. 1.
https://doi.org/10.1002/(SICI)1098-1136(199609)18:1<1::AID-GLIA1>3.0.CO;2-6

Bennett M.L., Bennett F.C., Liddelow S.A., Ajami B., Zamanian J.L., Fernhoff N.B., Mulinyawe S.B., Bohlen C.J., Adil A., Tucker A., Weissman I.L., Chang E.F., Li G., Grant G.A., Hayden Gephart M.G., Barres B.A. 2016. New tools for studying microglia in the mouse and human CNS. Proc. Natl. Acad. Sci. USA. V. 113. P. 1738.
https://doi.org/10.1073/pnas.1525528113

Bernal A., Arranz L. 2018. Nestin-expressing progenitor cells: function, identity and therapeutic implications. Cell. Mol. Life Sci. V. 75. P. 2177.
https://doi.org/10.1007/s00018-018-2794-z

Blasi E., Barluzzi R., Bocchini V., Mazzolla R., Bistoni F. 1990. Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. J. Neuroimmunol. V. 27. P. 229.
https://doi.org/10.1016/0165-5728(90)90073-V

Bohnert S., Seiffert A., Trella S., Bohnert M., Distel L., Ondruschka B., Monoranu C.M. 2020. TMEM119 as a specific marker of microglia reaction in traumatic brain injury in postmortem examination. Int. J. Legal Med. V. 134. P. 2167.
https://doi.org/10.1007/s00414-020-02384-z

Bonsack F., Sukumari-Ramesh S. 2018. TSPO: an evolutionarily conserved protein with elusive functions. Int. J. Mol. Sci. V. 19. P. 1694.
https://doi.org/10.3390/ijms19061694

Cornforth M.N. 2001. Analyzing radiation-induced complex chromosome rearrangements by combinatorial painting. Radiat. Res. V. 155. P. 643.
https://doi.org/10.1007/978-1-4939-9432-8_15

Coniglio S.J., Eugenin E., Dobrenis K., Stanley E.R., West B.L., Symons M.H., Segall J.E. 2012. Microglial stimulation of glioblastoma invasion involves epidermal growth factor receptor (EGFR) and colony stimulating factor 1 receptor (CSF-1R) signaling. Mol. Med. V. 18. P. 519.
https://doi.org/10.2119/molmed.2011.00217

Coskun V., Wu H., Blanchi B., Tsao S., Kim K., Zhao J., Biancotti J.C., Hutnick L., Krueger R.C. Jr., Fan G., de Vellis J., Sun Y.E. 2008. $CD133^+$ neural stem cells in the ependyma of mammalian postnatal forebrain. Proc. Natl. Acad. Sci. USA. V. 105. P. 1026.
https://doi.org/10.1073/pnas.0710000105

Douglas M.R., Morrison K.C., Jacques S.J., Leadbeater W.E., Gonzalez A.M., Berry M., Logan A., Ahmed Z. 2009. Off-target effects of epidermal growth factor receptor antagonists mediate retinal ganglion cell disinhibited axon growth. Brain. V. 132. P. 3102.
https://doi.org/10.1093/brain/awp240

Elmore M.R., Najafi A.R., Koike M.A., Dagher N.N., Spangenberg E.E., Rice R.A., Kitazawa M., Matusow B., Nguyen H., West B.L., Green K.N. 2014. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron. V. 82. P. 380.
https://doi.org/10.1016/j.neuron.2014.02.040

Eyo U.B., Dailey M.E. Microglia: key elements in neural development, plasticity, and pathology. 2013. J. Neuroimmune Pharmacol. V. 8. P. 494.
https://doi.org/10.1007/s11481-013-9434-z

Fischer O.M., Hart S., Ullrich A. 2006. Dissecting the epidermal growth factor receptor signal transactivation pathway. Methods Mol. Biol. V. 327. P. 85.
https://doi.org/10.1385/1-59745-012-X:85

Green K.N., Crapser J.D., Hohsfield L.A. 2020. To kill a microglia: A case for CSF1R inhibitors. Trends Immunol. V. 41. P. 771.
https://doi.org/10.1016/j.it.2020.07.001

Hagan N., Kane J.L., Grover D., Woodworth L., Madore C., Saleh J., Sancho J., Liu J., Li Y., Proto J., Zelic M., Mahan A., Kothe M., Scholte A.A., Fitzgerald M. 2020. CSF1R signaling is a regulator of pathogenesis in progressive MS. Cell Death Dis. V. 11. P. 904.
https://doi.org/10.1038/s41419-020-03084-7

Han J., Chitu V., Stanley E.R., Wszolek Z.K., Karrenbauer V.D., Harris R.A. 2022. Inhibition of colony stimulating factor-1 receptor (CSF-1R) as a potential therapeutic strategy for neurodegenerative diseases: opportunities and challenges.

Cell. Mol. Life Sci. V. 79. P. 219.
https://doi.org/10.1007/s00018-022-04225-1

Huang Y., Xu Z., Xiong S. 2018. Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion. Nat. Neurosci. V. 21. P. 530.
https://doi.org/10.1038/s41593-018-0090-8

Jenkins S.J., Ruckerl D., Thomas G.D., Hewitson J.P., Duncan S., Brombacher F., Maizels R.M., Hume D.A., Allen J.E. 2013. IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. J. Exper. Med. V. 210. P. 2477.
https://doi.org/10.1084/jem.20121999

Jones S., Rappoport J.Z. 2014. Interdependent epidermal growth factor receptor signalling and trafficking. Int. J. Biochem. Cell. Biol. V. 51. P. 23.
https://doi.org/10.1016/j.biocel.2014.03.014

Jurga A.M., Paleczna M., Kuter K.Z. 2020. Overview of general and discriminating markers of differential microglia phenotypes. Front. Cell Neurosci. V. 14. P. 198.
https://doi.org/10.3389/fncel.2020.00198

Kalm M., Andreasson U., Björk-Eriksson T., Zetterberg H., Pekny M., Blennow K., Pekna M., Blomgren K. 2016. C3 deficiency ameliorates the negative effects of irradiation of the young brain on hippocampal development and learning. Oncotarget. V. 7. P. 19382.
https://doi.org/10.18632/oncotarget.8400

Lei F., Cui N., Zhou C., Chodosh J., Vavvas D.G., Paschalis E.I. 2020. CSF1R inhibition by a small-molecule inhibitor is not microglia specific; affecting hematopoiesis and the function of macrophages. Proc. Natl. Acad. Sci. USA. V. 22. P. 23336.
https://doi.org/10.1073/pnas.1922788117

Lively S., Schlichter L.C. 2018. Microglia responses to pro-inflammatory stimuli (LPS, IFNγ + TNFα) and reprogramming by resolving cytokines (IL-4, IL-10). Front. Cell Neurosci. V. 12. P. 215.
https://doi.org/10.3389/fncel.2018.00215

Liu G.J., Middleton R.J., Hatty C.R., Kam W.W., Chan R., Pham T., Harrison-Brown M., Dodson E., Veale K., Banati R.B. 2014. The 18kDa translocator protein, microglia and neuroinflammation. Brain Pathol. V. 24. P. 631.
https://doi.org/10.1111/bpa.12196

Mansour H.M., Fawzy H.M., El-Khatib A.S., Khattab M.M. 2022. Repurposed anti-cancer epidermal growth factor receptor inhibitors: mechanisms of neuroprotective effects in Alzheimer's disease. Neural Regen. Res. V. 17. P. 1913.
https://doi.org/10.4103/1673-5374.332132

Michalczyk K., Ziman M. 2005. Nestin structure and predicted function in cellular cytoskeletal organisation. Histol. Histopathol. V. 20. P. 665.
https://doi.org/10.14670/HH-20.665

Muñoz-Garcia J., Cochonneau D., Télétchéa S., Moranton E., Lanoe D., Brion R., Lézot F., Heymann M.F., Heymann D. 2021. The twin cytokines interleukin-34 and CSF-1: masterful conductors of macrophage homeostasis. Theranostics. V. 11. P. 1568.
https://doi.org/10.7150/thno.50683

Nagamoto-Combs K., Kulas J., Combs C.K. 2014. A novel cell line from spontaneously immortalized murine microglia. J. Neurosci. Methods. V. 15. P. 187.
https://doi.org/10.1016/j.jneumeth.2014.05.021

Onyango I.G., Jauregui G.V., Čarná M., Bennett J.P. Jr., Stokin G.B. 2021. Neuroinflammation in Alzheimer's Disease. Biomedicines. V. 9. P. 524.
https://doi.org/10.3390/biomedicines9050524

Pannell M., Economopoulos V., Wilson T.C., Kersemans V., Isenegger P.G., Larkin J.R., Smart S., Gilchrist S., Gouverneur V., Sibson N.R. 2020. Imaging of translocator protein upregulation is selective for pro-inflammatory polarized astrocytes and microglia. Glia. V. 68. P. 280.
https://doi.org/10.1002/glia.23716

Prater K.E., Aloi M.S., Pathan J.L., Winston C.N., Chernoff R.A., Davidson S., Sadgrove M., McDonough A., Zierath D., Su W., Weinstein J.R., Garden G.A. 2021. A Subpopulation of microglia generated in the adult mouse brain originates from Prominin-1-expressing progenitors. J. Neurosci. V. 41. P. 7942.
https://doi.org/10.1523/JNEUROSCI.1893-20.2021

Qu W.S., Liu J.L., Li C.Y., Li X., Xie M.J., Wang W., Tian D.S. 2015. Rapidly activated epidermal growth factor receptor mediates lipopolysaccharide-triggered migration of microglia. Neurochem. Int. V. 90. P. 85.
https://doi.org/10.1016/j.neuint.2015.07.007

Qu W.S., Tian D.S., Guo Z.B., Fang J., Zhang Q., Yu Z.Y., Xie M.J., Zhang H.Q., Lü J.G., Wang W. 2012. Inhibition of EGFR/MAPK signaling reduces microglial inflammatory response and the associated secondary damage in rats after spinal cord injury. J. Neuroinflammation. V. 9. P. 178.
https://doi.org/10.1186/1742-2094-9-178

Ramprasad M.P., Terpstra V., Kondratenko N., Quehenberger O., Steinberg D. 1996. Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. Proc. Natl. Acad. Sci. USA. V. 93. P. 14833.
https://doi.org/10.1073/pnas.93.25.14833

Sasaki Y., Ohsawa K., Kanazawa H., Kohsaka S., Imai Y. 2001. Iba1 is an actin-cross-linking protein in macrophages/microglia. Biochem. Biophys. Res. Commun. V. 286. P. 292.
https://doi.org/10.1006/bbrc.2001.5388

Stanley E.R., Chitu V. 2014. CSF-1 receptor signaling in myeloid cells. Cold Spring Harb. Perspect Biol. V. 6. P. a021857.
https://doi.org/10.1101/cshperspect.a021857

Stansley B., Post J., Hensley K. 2012. A comparative review of cell culture systems for the study of microglial biology in Alzheimer's disease. J. Neuroinflammation. V. 9. P. 115.
https://doi.org/10.1186/1742-2094-9-115

Takamori Y., Mori T., Wakabayashi T., Nagasaka Y., Matsuzaki T., Yamada H. 2009. Nestin-positive microglia in adult rat cerebral cortex. Brain Res. V. 1270. P. 10.
https://doi.org/10.1016/j.brainres.2009.03.014

Waller R., Baxter L., Fillingham D.J., Coelho S., Pozo J.M., Mozumder M., Frangi A.F., Ince P.G., Simpson J.E., Highley J.R. 2019. Iba-1-/CD68$^+$ microglia are a prominent feature of age-associated deep subcortical white matter lesions. PLoS One. V. 25. P. e0210888.
https://doi.org/10.1371/journal.pone.0210888

Wohl S.G., Schmeer C.W., Friese T., Witte O.W., Isenmann S. 2011. In situ dividing and phagocytosing retinal microglia express nestin, vimentin, and NG2 in vivo. PLoS One.

V. 6. P. e22408.
https://doi.org/10.1371/journal.pone.0022408

Wong A.M., Patel N.V., Patel N.K., Wei M., Morgan T.E., de Beer M.C., de Villiers W.J., Finch C.E. 2005. Macrosialin increases during normal brain aging are attenuated by caloric restriction. Neurosci. Lett. V. 390. P. 76.
https://doi.org/10.1016/j.neulet.2005.07.058

Yan P., Wu X., Liu X., Cai Y., Shao C., Zhu G. 2019. A Causal relationship in spinal cord injury rat model between microglia activation and EGFR/MAPK detected by overexpression of microRNA-325-3p. J. Mol. Neurosci. V. 68. P. 181.
https://doi.org/10.1007/s12031-019-01297-w

Yang Y., Sun Y., Hu R., Yan J., Wang Z., Li W., Jiang H. 2021. Morphine promotes microglial activation by upregulating the EGFR/ERK signaling pathway. PLoS One. V. 16 P. e0256870.
https://doi.org/10.1371/journal.pone.0256870

Microglia Cell Line SIM-A9 Features – New Data

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SIM-A9 is a line of spontaneously immortalized mouse microglia cells obtained from newborn C57BL/6 mice's cerebrum. The aim of this work is to characterize SIM-A9 line by the ratio of cells with the resting and activated microglia phenotype, to analyze the expression of stem/progenitor cell markers CD133 and nestin, growth factors receptors CSF-1R and EGFR, and the karyotype of this line. The light microscopy, immunocytochemistry, flow cytometry and RT/PCR were used to analyze the morphology, phenotype, and gene expression levels of pro-inflammatory cytokines, and the mFISH method was used to analyze the karyotype. It was shown for the first time that SIM-A9 cells express a high level of TSPO protein, CD68, CD11b and CD45 markers on the surface membrane of cells, which corresponds to the phenotype of activated microglia. Despite this, the cells of this line respond with additional activation to LPS stimulation, which leads to an increase in the pro-inflammatory cytokine genes IL-1β, TNFα, IL-6 expression and a high level of active oxygen and nitrogen metabolites formation. It was shown that SIM-A9 cells express stem and progenitor cells markers, $CD133^+$ and nestin, which allows us to consider the cells of this line as early poorly differentiated progenitor cells, despite their phenotype corresponding to activated microglia. It was also found that SIM-A9 cells express receptors of two growth factors CSF-1 and EGF, CSF-1R and EGFR, which indicates the possibility of SIM-A9 cells proliferation stimulation by two alternative mechanisms under the action of the corresponding factors. SIM-A9 cells have a hypotetraploid karyotype with a large number of structural and quantitative chromosome anomalies.

Keywords: microglia, cell line SIM-A9, karyotype, stem cells, CSF-1 receptor, EGF receptor, gene expression, cytokines