ISSN 2079-0570, Advances in Gerontology, 2021, Vol. 11, No. 2, pp. 126–131. © Pleiades Publishing, Ltd., 2021.

Sirtuins Expression in the Hippocampus and Buccal Epithelium of Elderly and Senile Individuals with Alzheimer's Disease

A. E. Pukhalskaia[a, *], N. S. Linkova[a, b, c], A. S. Diatlova[a], K. L. Kozlov[a], I. M. Kvetnoy[a, d, e], M. V. Koroleva[b], and A. M. Volkov[f]

[a] *St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, 197110 Russia*
[b] *Academy of Postgraduate Education, Moscow, 125371 Russia*
[c] *Belgorod National Research University, Belgorod, 308015 Russia*
[d] *St. Petersburg State University, St. Petersburg, 199034 Russia*
[e] *St. Petersburg Research Institute of Phthisiopulmonology, St. Petersburg, 191036 Russia*
[f] *Kirov Military Medical Academy, St. Petersburg, 194044 Russia*
**e-mail: ibg@gerontology.ru*

Received April 6, 2020; revised April 21, 2020; accepted April 29, 2020

Abstract—Sirtuins (SIRT) are a class of NAD-dependent proteins with deacetylase activity that are involved in the regulation of transcription, metabolic pathways, and cell aging via the deacetylation of histone and nonhistone targets. It was hypothesized that sirtuins play important role in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD). An age-related decrease in sirtuin expression leads to oxidative stress, which can cause neurodegeneration. This article examines the age-related dynamics of SIRT1, 3, 5, and 6 expression in patients with AD and in healthy individuals with immunohistochemistry and immunocytochemistry methods via immunofluorescent confocal microscopy. In elderly and senile health individuals, the SIRT1, 3, 5, and 6 expression in the hippocampus and buccal epithelium did not differ significantly. In AD patients, the SIRT1, 3, 5, and 6 expression in the hippocampus and buccal epithelium decrease by 1.5–5 times as compared with healthy elderly and senile individuals. The SIRT5 expression in the hippocampus and buccal epithelium does not depend on the age or AD diagnosis. Thus, the SIRT1, 3, and 6 expression in the buccal epithelium can be a marker for intravital, noninvasive AD diagnosis in elderly and senile individuals.

Keywords: sirtuins, Alzheimer's disease, hippocampus, buccal epithelium, intravital diagnosis

DOI: 10.1134/S2079057021020120

INTRODUCTION

Sirtuins (silent information regulator, SIRT1–7) are a family of seven evolutionarily conserved, nicotinamide adenine dinucleotide (NAD)-dependent proteins. Their ability to regulate transcription and cellular senescence healthy via deacetylation of histone targets was first to be discovered [9]. Later studies expanded the understanding of the functions of sirtuins and made it possible to establish that they have the ability to deacylate nonhistone proteins as well [3, 19]. Sirtuins are found in all living organisms and are involved in the regulation of metabolic pathways and the epigenetic regulation of gene expression. SIRT1, 2, 6, and 7 in mammals are located in the nucleus, SIRT1 and 2 are found in the cytoplasm, and SIRT3, 4, and 5 are located in mitochondria [21].

In 2017–2018 it became evident that sirtuins play an important role in the development of neurodegenerative diseases, in particular, Alzheimer's disease (AD). In mice with reduced SIRT6 expression, the level of brain-neuron apoptosis and the expression of the hyperphosphorylated form of the τ-protein increased and signs of neurodegeneration were observed. SIRT6 expression was also decreased in AD patients [10]. A decrease in SIRT3 expression in animals and humans leads to activation of the expression of the τ-protein and the Aβ42 peptide—proteins that are involved in the pathogenesis of AD [22]. The suppression of SIRT1 expression in transgenic mice with AD leads to activation of the synthesis of amyloid peptide Aβ42 and oxidative stress. A similar pattern was observed in patients with asthma [2]. It was suggested that the assessment of the sirtuin synthesis in peripheral tissues may be important for the diagnosis and assessment of the effectiveness of AD therapy [16].

The goal of this work was to study the content of SIRT1, 3, 5, and 6 in the hippocampus and buccal epithelium in elderly and senile patients without neuropathology and in those with AD.

EXPERIMENTAL

The buccal epithelium (BE) of "healthy" donors (58 individuals without neuropathology, the control group) and patients with AD (64 individuals) was obtained at St. George's Hospital (St. Petersburg). They were elderly (63 ± 2.4 years) and senile (82 ± 2.3 years) individuals. Autopsy material from the hippocampus was also obtained there from elderly (48 individuals, 67 ± 2.3 years) and senile (56 individuals, 81 ± 2.1 years) individuals without neuropathology and from elderly (52 individuals, 68.5 ± 2 years) and senile (58 individuals, 84 ± 2.5 years) patients with AD. The BE was collected with the written consent of the patients or their relatives. Individuals of the control group were assigned to the category of almost healthy individuals in accordance with the age norm based on clinical, laboratory, and instrumental examination. They did not have any diseases of the nervous, cardiovascular, respiratory, or endocrine systems in the exacerbation phase. All patients at the time of participation in the study were examined by a dentist and did not have diseases of the dentoalveolar system. During the study period, all participants received standard meals and were in a free regimen with their usual level of physical activity. The study was carried out in the autumn and winter period (November and December 2019). Patients with AD were diagnosed with an initial or moderate degree of dementia. The number of patients with initial or moderate dementia was the same in the studied age groups. The patients included in the study met the international criteria for the diagnosis of AD: ICD-10 (International Classification of Diseases) and NINCDS/ADRDA.

The BE was taken from the oral cavity (buccal mucosa) no earlier than 4 h after food consumption and rinsing of the mouth with saline. It was obtained with sterile disposable probes with synthetic hair, which was placed in a sterile disposable Eppendorf tube with a transport medium. Cytological smears were prepared with via liquid cytology with the Novoprep automated system (NRS, France).

Pieces of the hippocampus with a volume of 1 cm^3 were fixed in 10% neutral buffered formalin (pH 7.2), dehydrated with a Leica TP1020 automatic material transfer station, and embedded in paraffin. Paraffin sections 4–6 μm thick were placed on glass slides covered with a poly-L-lysine film (Sigma, United States) for subsequent immunohistochemical study.

Immunofluorescence Confocal Microscopy

Immunofluorescence confocal microscopy was performed on BE cells and paraffin sections of the hippocampus. Preparations of the hippocampus and BE were stained according to the standard protocol with primary antibodies to SIRT1 (1 : 100, Abcam), SIRT3 (1 : 50, Abcam), SIRT5 (1 : 100, Abcam), and SIRT6 (1 : 100, Dako). As secondary antibodies, we used antibodies conjugated with fluorophores: Alexa Fluor 567 (1 : 1000, Abcam, United States), Alexa Fluor 488 (1 : 1000, Abcam, United States), and Alexa Fluor 555 (1 : 1000, Abcam, United States). The cell nuclei were stained with Hoechst 33258 reagent (Sigma, United States). The obtained preparations were analyzed with an Olympus Fluoview CM FV300-IX70 inverted confocal microscope.

Morphometry

The results of immunocytochemical and immunohistochemical staining were assessed in a morphometric study based on IntelPentium 5 and the Videotest-Morphology 5.2 software with a computer analysis system for microscopic images that consisted of an Olympus Fluoview CM FV300-IX70 microscope, an Olympus digital camera, and a personal computer. In each case, five fields of vision were analyzed [1].

The expression area is a parameter calculated in the VideotestMorphology 5.2 program that characterizes the area of the immunopositive (stained) portion of cells. The information on the average cell number and size from one micrograph of a series of evaluated images was uploaded into the program. The relative area of expression occupied by immunopositive cells in relation to the total average area of cells in the field of view is calculated as a percentage. For markers with cytoplasmic staining, the expression area was estimated as the ratio of the area of the stained cytoplasm to the total cell area. For markers with nuclear staining, the expression area was calculated as the ratio of the area occupied by immunopositive nuclei to the total area of the nuclei in the field of view.

Statistical Analysis

The results were analyzed with the SPSS Statistics 17.0 software. The character of the distribution of indices was determined with the Kolmogorov–Smirnov test. The differences between the samples were assessed with the parametric Student's *t*-test for a normal distribution of data and the nonparametric Mann–Whitney *U*-test in the absence of a normal distribution of data. The critical level of reliability of the null statistical hypothesis (about the absence of significant differences) was taken to be equal to 0.01.

RESULTS AND DISCUSSION

Sirtuins expression in autopsy material from the hippocampus of healthy individuals and patients with alzheimer's disease of different ages. The area of *SIRT*1 expression in the hippocampus in elderly and senile individuals did not differ significantly. However, in elderly patients with AD, the SIRT1 expression area in the hippocampus was 3.5 times lower than that in the control group and elderly patients—it was five times lower than that in the control group (Figs. 1, 2). The

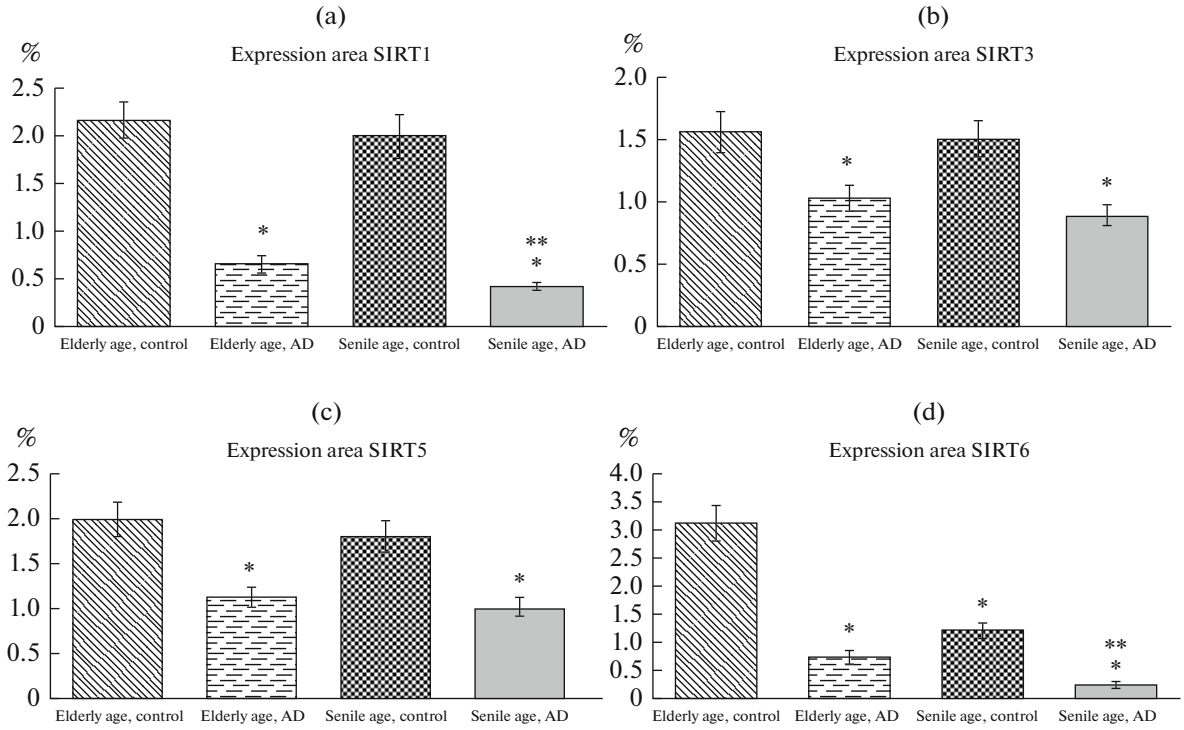

Fig. 1. Sirtuin expression in the hippocampus of healthy individuals and patients with Alzheimer's disease of different ages. (a) Area of SIRT1 expression, %; (b) area of SIRT3 expression, %; (c) area of SIRT5 expression, %; (d) area of SIRT6 expression, %. * $p < 0.01$ as compared with the control group in individuals of the corresponding age; ** $p < 0.01$ as compared with the elderly group with AD.

expression area of SIRT3, SIRT5, and SIRT6 in the hippocampus did not significantly differ in elderly and senile individuals without neuropathology, but there were marked differences between the AD and control groups. Thus, the SIRT3 expression area in the hippocampus of elderly patients with AD was 1.5 times lower than the corresponding control value; among individuals of senile age, it was 1.7 times lower. The SIRT5 expression area in elderly and senile patients with AD was 1.8 times lower than that in the control group. The SIRT6 expression area was 4.4 times lower in elderly patients with AD as compared to the corresponding indicator in individuals without neuropathology and six times lower in senile patients with AD as compared with the corresponding control. In addition, the expression area of SIRT1 and SIRT6 in the hippocampus of senile patients with AD was 1.75 and 2.8 times lower than that in elderly individuals with AD.

Sirtuin expression in the buccal epithelium of healthy individuals and patients with alzheimer's disease of different ages. The expression area of SIRT1, 3, 5, and 6 in the BE of elderly and senile individuals did not differ significantly. In elderly patients with AD, the SIRT1 expression area in the BE was three times lower than that in the control group, and it was 2.4 times lower in AD patients of senile age than that in individuals without neuropathology. The SIRT3 expression area in the BE in elderly AD patients was 2.5 times lower than the corresponding control value and was 3.1 times lower than the control in senile individuals. The SIRT5 expression area in the BE in elderly and senile AD patients did not differ significantly from this index in individuals without neuropathology. The SIRT6 expression area in the BE was 3.7 times lower in elderly AD patients as compared to the corresponding control index and 4.1 times lower in senile AD patients as compared with individuals without neuropathology (Figs. 3, 4). In addition, the SIRT6 expression area in the BE in senile AD patients was three times lower than that in elderly AD patients.

It is known that sirtuins are important regulators of redox homeostasis in cells; they modulate key genes and mechanisms of oxidative stress and regulate the expression and activity of antioxidant enzymes and the production of prooxidants. The pathophysiology of neurodegenerative diseases, including AD, is associated with oxidative stress and an increased synthesis of reactive oxygen species (ROS), which lead to neuronal damage and impairment of neuroplasticity [4, 5]. Brain tissue is more susceptible to oxidative stress due to its active oxygen consumption, low regeneration rate, high content of polyunsaturated fatty acids, and low antioxidant concentration [20].

Mitochondria damaged by oxidative stress can produce increased levels of ROS, which damage proteins, nucleic acids, and cell membranes and cause lipid per-

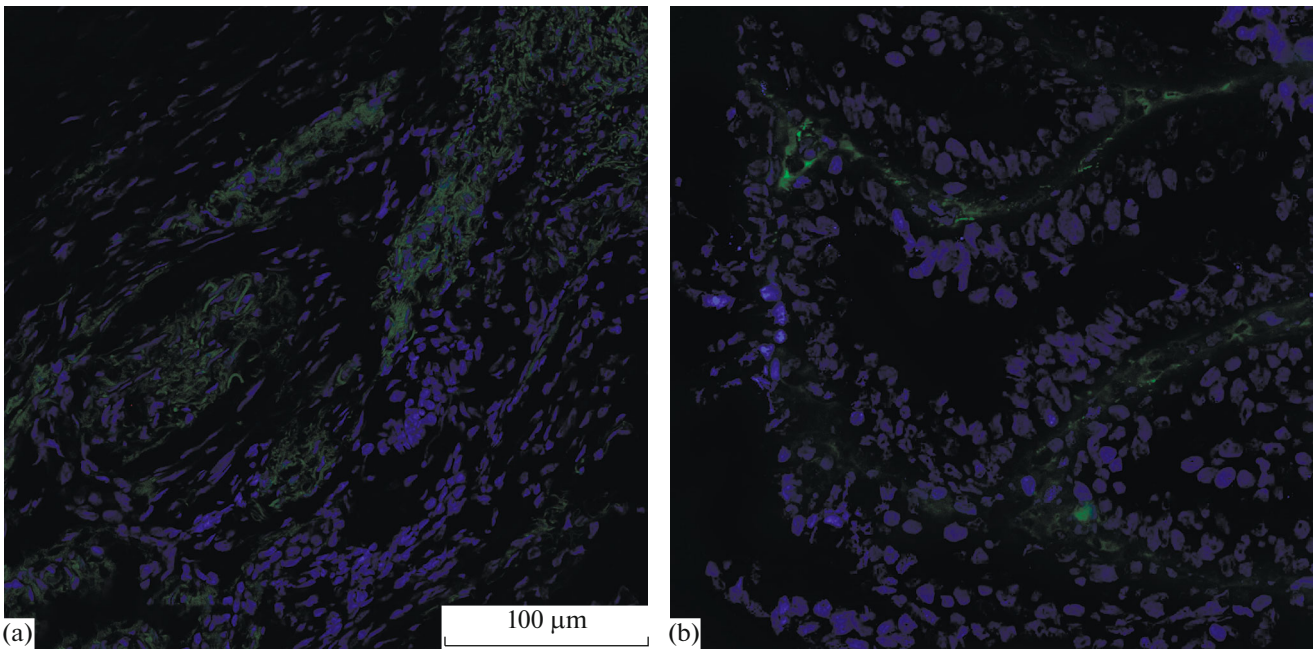

Fig. 2. Expression of sirtuin 1 in the hippocampus of the elderly individuals: (a) control and (b) AD. Confocal microscopy, 100×; immunofluorescent staining of the hippocampus with antibodies to sirtuin 1 (Alexa Fluor 488—green fluorescence); nuclei stained with Hoest 33258—blue fluorescence.

oxidation and increased permeability for calcium ions across the neuron membrane. Reactive oxygen species also increase the production of Aβ peptides [15].

A decrease in the level of sirtuins, in particular, SIRT1, was associated with an increase in the production and accumulation of amyloid Aβ in AD patients [8]. SIRT1 can regulate Aβ metabolism via modulation of the processing of the amyloid precursor protein (APP), and knockout of the SIRT1 gene correlates with increased Aβ production [11]. Overexpression of SIRT1 decreases the content of ROS and nitric oxide in the cell, the production of proinflammatory cytokines, and the expression of Aβ in the brain of AD patients [11, 13, 14, 17].

It has been shown that the Aβ expression in the mouse brain depends on the level of SIRT3 expression [18, 22]. SIRT3 dysregulation is associated with mitochondrial dysfunction in AD [12]. SIRT5 (mitochondrial) and SIRT6 (nuclear) are also considered to be signaling molecules that are involved in brain aging and the development of neurodegenerative diseases [7].

The immunohistochemical study of sirtuin expression in autopsy material from the hippocampus demonstrated that the content of SIRT1, 3, 5, and 6 in the hippocampal cells decreases by 1.5–5 times on average in AD. An age-related dynamics is also observed for the expression of SIRT1 and SIRT6 in the hippocampus. In the hippocampus of senile AD patients, their content is on average lower than that in the hippocampus of elderly patients with this neurodegenerative disease. The differences in SIRT1 expression in the hippocampus between the control group and AD patients were statistically significant, which confirms the available literature data on the possible role of SIRT1 in AD pathogenesis.

We have not found studies on sirtuin expression in the BE in the literature; however, there are data on SIRT1 expression in peripheral-blood lymphocytes of AD patients. The SIRT1 expression in peripheral-blood lymphocytes of AD patients is four times lower than that in individuals without neuropathology. Interestingly, this relationship persists for SIRT1 expression in the hippocampus, which was studied in this work [6].

CONCLUSIONS

We found that the sirtuin expression in the BE of individuals without neuropathology and in AD patients is subject to a similar relationship. In AD, the content of SIRT1, 3, and 6 in the BE decreases by an average of 2.5–4.1 times. For SIRT6, an age-related dynamics was observed: in the BE of senile AD patients, their content was, on average, three times lower than that in elderly patients with this pathology. However, SIRT5 expression in the BE did not depend on either age or the presence of AD in patients.

The expression of SIRT1, 3, and 6 in the BE in control group and in AD reflects its level in the hippocampus. The study of sirtuins in the BE may be a new promising approach for noninvasive, intravital diagnostics of AD in elderly and senile patients.

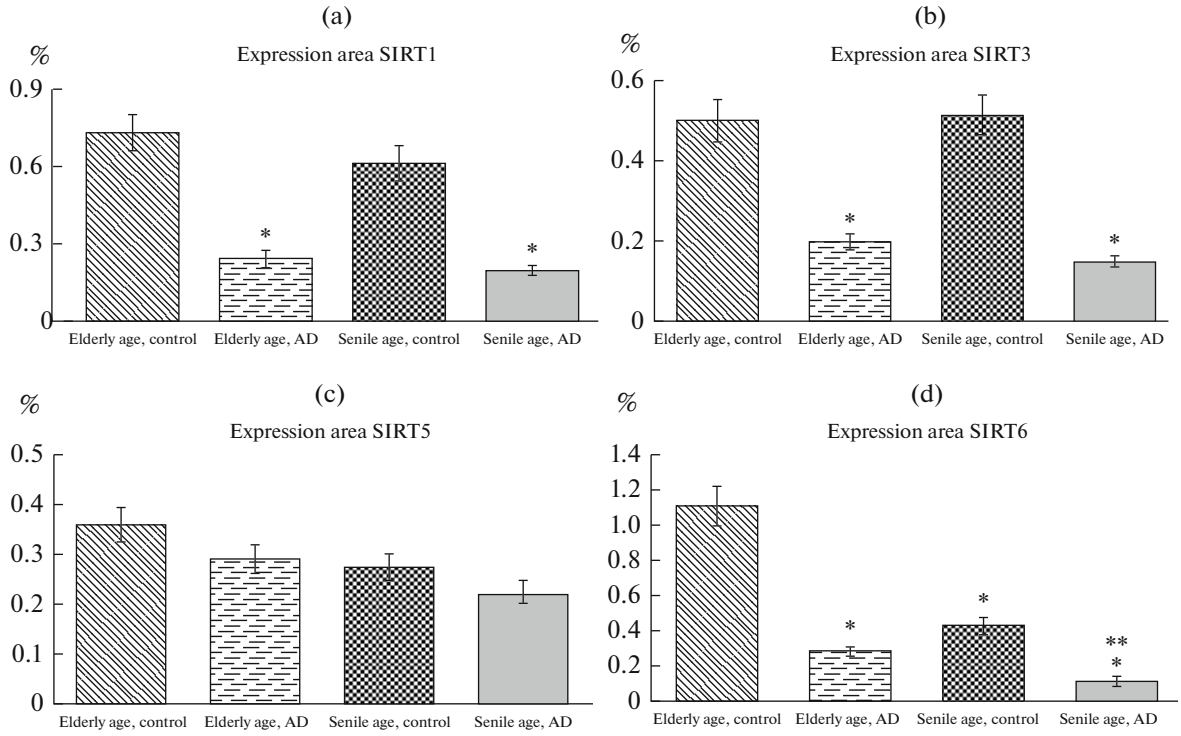

Fig. 3. Sirtuin expression in the buccal epithelium of healthy individuals and patients with Alzheimer's disease of different ages. (a) SIRT1 expression area, %; (b) SIRT3 expression area, %; (c) SIRT5 expression area, %; (d) *SIRT*6 expression area, %. * $p < 0.01$ as compared with the control group in individuals of the corresponding age; ** $p < 0.01$ as compared with the elderly group with AD.

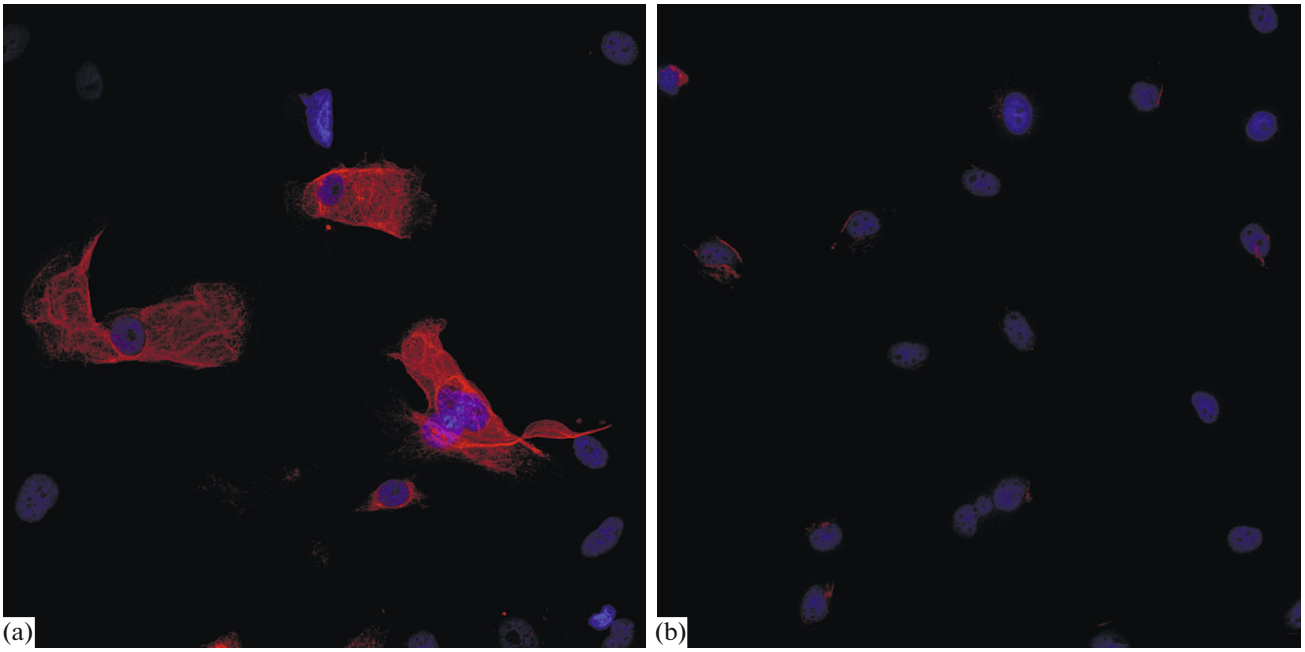

Fig. 4. Expression of sirtuin 6 in the buccal epithelium of elderly individuals: (a) control and (b) AD. Confocal microscopy, 200×; immunofluorescent staining of the buccal epithelium with antibodies to sirtuin 6 (Alexa Fluor 567—red fluorescence); nuclei stained with Hoest 33258—blue fluorescence.

FUNDING

This work was supported by the Russian Foundation for Basic Research (project no. 18-54-06012 Az_a).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

REFERENCES

1. Avtandilov, G.G., *Meditsinskaya morfometriya: Rukovodstvo* (Medical Morphometry: A Handbook), Moscow: Meditsina, 1990.
2. Cao, W., Dou, Y., and Li, A., Resveratrol boosts cognitive function by targeting SIRT1, *Neurochem. Res.,* 2018, vol. 43, no. 9, pp. 1705–1713. https://doi.org/10.1007/s11064-018-2586-8
3. Chen, B., Zang, W., Wang, J., et al., The chemical biology of sirtuins, *Chem. Soc. Rev.,* 2015, vol. 44, pp. 5246–5264.
4. Fang, C., Gu, L., Smerin, D., et al., The interrelation between reactive oxygen species and autophagy in neurological disorders, *Oxid. Med. Cell. Longevity,* 2017, vol. 2017, art. ID 8495160. https://doi.org/10.1155/2017/8495160
5. Gomes, B.A.Q., Silva, J.P.B., Romeiro, C.F.R., et al., Neuroprotective mechanisms of resveratrol in Alzheimer's disease: role of SIRT1, *Oxid. Med. Cell. Longevity,* 2018, vol. 2018, art. ID 8152373. https://doi.org/10.1155/2018/8152373
6. Hadar, A., Milanesi, E., Walczak, M., et al., SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's disease, *Sci. Rep.,* 2018, vol. 8, no. 1, p. 8465. https://doi.org/10.1038/s41598-018-26547-6
7. Jęśko, H., Wencel, P., Strosznajder, R.P., and Strosznajder, J.B., Sirtuins and their roles in brain aging and neurodegenerative disorders, *Neurochem. Res.*, 2017, vol. 42, no. 3, pp. 876– 890. https://doi.org/10.1007/s11064-016-2110-y
8. Julien, C., Tremblay, C., Emond, V., et al., Sirtuin 1 reduction parallels the accumulation of tau in Alzheimer disease, *J. Neuropathol. Exp. Neurol.,* 2009, vol. 68, no. 1, pp. 48–58. https://doi.org/10.1097/NEN.0b013e3181922348
9. Kaeberlein, M., McVey, M., and Guarente, L., The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms, *Genes Dev.,* 1999, vol. 13, pp. 2570–2580.
10. Kaluski, S., Portillo, M., Besnard, A., et al., Neuroprotective functions for the histone deacetylase SIRT6, *Cell Rep.,* 2017, vol. 18, no. 13, pp. 3052–3062. https://doi.org/10.1016/j.celrep.2017.03.008
11. Koo, J.H., Kang, E.B., Oh, Y.S., et al., Treadmill exercise decreases amyloid-β burden possibly via activation of SIRT-1 signaling in a mouse model of Alzheimer's disease, *Exp. Neurol.,* 2017, vol. 288, pp. 142–152. https://doi.org/10.1016/j.expneurol.2016.11.014
12. Lee, J., Kim, Y., Liu, T., et al., SIRT3 deregulation is linked to mitochondrial dysfunction in Alzheimer's disease, *Aging Cell,* 2018, vol. 17, no. 1, p. e12679. https://doi.org/10.1111/acel.12679
13. Marwarha, G., Raza, S., Meiers, C., and Ghribi, O., Leptin attenuates BACE1 expression and amyloid-β genesis via the activation of SIRT1 signaling pathway, *Biochim. Biophys. Acta, Mol. Basis Dis.*, 2014, vol. 1842, no. 9, pp. 1587–1595. https://doi.org/10.1016/j.bbadis.2014.05.015
14. Morris-Blanco, K.C., Cohan, C.H., Neumann, J.T., et al., Protein kinase C epsilon regulates mitochondrial pools of Nampt and NAD following resveratrol and ischemic preconditioning in the rat cortex, *J. Cereb. Blood Flow Metab.,* 2014, vol. 34, no. 6, pp. 1024–1032.
15. Palacino, J.J., Sagi, D., Goldberg, M.S., et al., Mitochondrial dysfunction and oxidative damage in *parkin*-deficient mice, *J. Biol. Chem.,* 2004, vol. 279, no. 18, pp. 18614–18622.
16. Rizzi, L. and Roriz-Cruz, M., Sirtuin 1 and Alzheimer's disease: an up-to-date review, *Neuropeptides,* 2018, vol. 71, pp. 54–60. https://doi.org/10.1016/j.npep.2018.07.001
17. Salminen, A., Kaarniranta, K., and Kauppinen, A., Crosstalk between oxidative stress and SIRT1: impact on the aging process, *Int. J. Mol. Sci.,* 2013, vol. 14, no. 2, pp. 3834–3859.
18. Salvatori, I., Valle, C., Ferri, A., and Carrì, M.T., SIRT3 and mitochondrial metabolism in neurodegenerative diseases, *Neurochem. Int.,* 2017, vol. 109, pp. 184–192. https://doi.org/10.1016/j.neuint.2017.04.012
19. Singh, C.K., Chhabra, G., Ndiaye, M.A., et al., The role of sirtuins in antioxidant and redox signaling, *Antioxid. Redox Signaling,* 2018, vol. 28, no. 8, pp. 643–661. https://doi.org/10.1089/ars.2017.7290
20. Tönnies, E. and Trushina, E., Oxidative stress, synaptic dysfunction, and Alzheimer's disease, *J. Alzheimer's Dis.,* 2017, vol. 57, no. 4, pp. 1105–1121. https://doi.org/10.3233/JAD-161088
21. Xu, S., Bai, P., and Jin, Z.G., Sirtuins in cardiovascular health and diseases, *Trends Endocrinol. Metab.,* 2016, vol. 27, no. 10, pp. 677–678. https://doi.org/10.1016/j.tem.2016.07.004
22. Yin, J., Li, S., Nielsen, M., et al., Sirtuin 3 attenuates amyloid-β induced neuronal hypometabolism, *Aging* (New York), 2018, vol. 10, no. 10, pp. 2874–2883. https://doi.org/10.18632/aging.101592

Translated by A. Kashevarova