DOI 10.18699/SBB-2022-635

Analysis of changes in microglia and cerebral endothelium in hippocampus of OXYS rats during progression of Alzheimer's disease-like pathology

Peunov D.A.[1, 2]*, Tyumentsev M.A.[1], Rudnitskaya E.A.[1]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Novosibirsk State University, Novosibirsk, Russia*

** d.peunov@g.nsu.ru*

Key words: Alzheimer's disease, microglia, cerebral endothelium, OXYS rats

Motivation and Aim: Sporadic Alzheimer's disease (AD) is the most spread neurodegenerative disorder that causes dementia in elder people. Among all brain structures the hippocampus is one that suffers most and undergoes the earliest pathological changes. There is growing evidence for the pivotal role of neuroinflammation and dysfunction of cerebral endothelium (CE) in AD. Among brain cells microglia–local phagocytes of the central nervous system (CNS)– are assumed to be the main driver of inflammation. In its turn inflammation staying unresolved for a long time may harm other cells including CE. In the case of AD this damage causes a lack in supplementation of brain with trophic substrates, worsening of amyloid-beta (Aß) clearance as well as uncontrollable entrance of factors provoking inflammation. A feedback interaction between proinflammatory microglial activity and cerebral vasculature function appears to exist, and its reveal demands a presence of adequate AD models. Senescence-accelerated OXYS rats are considered as one of such models.

Methods and Algorithms: The work was performed using OXYS and Wistar rats (maternal strain used as a control). To reveal AD-associated changes we tested animals at the age of 20, 45 days; 3, 5, 12 and 18 months. The quantity and activation state of microglia were assessed by immunohistochemistry ($n = 6$). Occludin level was measured by western-blot analysis ($n = 6$). Structure of CE was imaged by electron microscopy. For the gene set obtained by intersection of differently expressed in OXYS rats (compared to Wistar rats) genes [1] with microglia– or CE–enriched genes [2] over representation analysis was conducted

Results: We showed that OXYS rats in comparison with Wistar rats had higher total and activated microglia density at all ages which for activated microglia was mostly pronounced at 45 days. Upon the results of transcriptome analysis at 20 days OXYS rats had altered expression level of 10 microglia-enriched genes. Among them the biological substrate oxidation pathway was presented (*Cyp4v3*↑, *Paox*↓, *Ptgs1*↓, FDR<0.05). At the 5 months of age expression levels of 4 genes were altered. Among them *Map3k14*↑ – the NF-kβ signaling pathway related gene was present. Also no differences in expression of proinflammatory genes downregulated in OXYS rats earlier were detected. At the18 months of age expression levels of 33 genes were altered. Among these genes the PI3K-AKT↓ (*Pik3cg, Spp1, Cdk6*), TNF↑ (*Map3k14, Cebpb, Junb*) signaling pathways and genes related to protein ubiquitinilation (*Nhlrc3, Ubc*), carbohydrates metabolism (*Hk2, Slc2a5, Mlxipl*) were altered. The occludin level in OXYS rats was lower than in Wistar rats at all ages, being significantly lower at 45 days and trending

downward at 4 months of age. That could be a sign for disruption of tight junctions and was also supported by electron microscopy studies. At 20 days of age we observed 8 CE enriched genes being differently expressed in OXYS rats. Among them genes related to lipid metabolism (*Sgms2*↓, *Hmgcs2*↑), transendothelial transport (*Cav2*↑) and reaction to immune activity (*Sod3*↑, *St3gal6*↑) were presented. At 5 months of age we detected 12 differently expressed genes. Among them genes related to the WNT(*Fzd6*↓) and NOTCH (*Dll4*↑, *Egfl8*↑) were presented. At 18 months of age OXYS rats had 43 differently expressed genes. Among them biological processes namely – blood circulation, negative regulation of cell development, negative regulation of stimuli response, adhesive contacts, regulation of adhesive contacts formation, biosynthesis of prostaglandins and metabolism of sphingolipids – were altered.

Conclusion: At the age prior to the manifestation of AD-like pathology (20-45 days) in OXYS rats presumably signs of CE disruption take place (*Cav2*↑). Also transcriptome analysis results indirectly point to the immunoactive milieu enriched with reactive oxygen species (*Sod3*↑, *St3gal6*↑). Concurrently according to the results of immunohistochemistry microglia appear to be slightly more active in OXYS rats at this age. However analysis of microglia-enriched genes showed diminished levels of some inflammation-associated genes (*Paox*↓, *Ptgs1*↓). Together these observations could indicate that activation of microglia is consequent to the disturbance of CE. Suppressed microglial pro-inflammatory response to CE impalement could point to their dysfunction or represent the recovery after preexisting inflammation. At the age when AD signs begin to manifest (3-5 months) we still observe signs of tight junctions disturbance in OXYS rats although signs of direct damage of CE are absent. At the same time the alterations in the growth-related signaling pathways could point to the suppression of angiogenesis. Also the *Fzd6* gene encodes the receptor that mainly activates canonical and planar cell polarity pathways which in turn oppose the WNT/Ca^{2+} pathway which may enhance inflammation. Hence suppression of the FZD6/WNT branch could be the factor promoting inflammation induction. Microglia phenotype at this age appears to be rather neutral or slightly pro-inflammatory according to the transcriptional analysis. At the age of active AD-like pathology progression and Aß accumulation (18 months) we observe signs of Aß metabolism disturbance both in microglia (*Nhlrc3*↓, *Ubc*↓) and CE (*Abcab1*). At the same time, CE appears to undergo endothelial-to-mesenchimal transformation and has weakened connection with the extracellular matrix. Microglia at this age seem to express a wider (compared to the age of 5 months) spectrum of proinflammatory genes at higher level. It's known that inflammation could promote all listed adverse alterations of CE. Ultimately in OXYS rats dysfunction of CE could be an early trigger of pathogenesis development but microglial activation at this time may exert cytoprotective effect while later microglial proinflammatory activity presumably promotes worsening of CE pathology that leads to progression of AD-like pathology.

Acknowledgements: The research was supported by RSF (No. 19-15-00044).

References

1. Stefanova N.A., Ershov N.I., Kolosova N.G. Suppression of Alzheimer's Disease-Like Pathology Progression by Mitochondria-Targeted Antioxidant SkQ1: A Transcriptome Profiling Study. *Oxid Med Cell Longev*. 2019;2019:3984906. doi: 10.1155/2019/3984906.
2. Zhang Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014;34(36)11929-11947.