

ABSTRACT

PHAGOCYTE BIOLOGY

P002-T | ARHGAP25 is a crucial player in the pathomechanism of rheumatoid arthritis in mice

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Background: ARHGAP25, a GTP-ase activating protein for Rac is mainly expressed in hematopoietic cells and plays predominant role in regulation of neutrophilic effector functions (phagocytosis, superoxide production), as well as neutrophil recruitment and extravasation due to its effect on Rac-dependent cytoskeletal changes. These findings strongly suggest, that ARHGAP25 may be involved in regulation of inflammatory diseases as well. This led us to investigate its role in autoantibody-induced model of rheumatoid arthritis, in transgenic mice.

Material and methods: After intraperitoneal injection of K/B×N mouse strain-derived serum into Arhgap25^{-/-} (KO) and wild type (WT) mice, ankle thickness was measured and a clinical score, indicating the severity of inflammation was determined. The loss of function was investigated by hanging on the mice on a wire-grid. Neutrophil infiltration into the ankle joints and the amount of filamentary actin in infiltrated neutrophils were measured with flow cytometry, using KO or WT mice.

Results: Absence of ARHGAP25 caused a remarkable decrease in clinical scores, as well as in ankle thickness compared to wild type mice. Similar results were observed in the functional test: ARHGAP25^{-/-} animals spent longer time on the grid. Moreover, lack of ARHGAP25 significantly reduced the infiltrated neutrophil count in ankle joints and F-actin amount in infiltrated neutrophils compared to WT. In mice treated with control serum neither inflammation, nor loss of function or elevated neutrophil infiltration could be observed, regardless of the genotype.

Conclusions: Our results suggest, that lack of ARHGAP25 result in the reduction of the signs of inflammation, as well as the neutrophil count in arthritic ankle joints, which may be linked to the altered actin-reorganization. Our current results indicate, that beyond the elementary phagocyte functions, ARHGAP25 is a crucial player of inflammation in a human disease-related complex model.

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P003-T | Altered homeostasis of peripheral neutrophils correlates with Alzheimer's disease progression

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Background: Alzheimer's disease, the most common form of dementia in the elderly, is characterized by a triad of pathological features: extracellular amyloid deposits predominantly composed of amyloid-β peptides, intracellular neurofibrillary tangles made of hyper- and abnormally phosphorylated Tau protein, and gliosis. Recent studies have underlined the effect of systemic inflammation on the pathophysiology of Alzheimer's disease. Neutrophils are key components of early innate immunity and contribute to uncontrolled systemic inflammation if not tightly regulated. The aim of our study was to fully characterize human circulating neutrophils at different disease stages in Alzheimer's disease.

Material and methods: We analyzed neutrophil phenotypes and functions in forty-two patients with Alzheimer's disease (sixteen with mild cognitive impairment and twenty-six with dementia), and compared them with twenty-two age-matched healthy subjects. This study was performed directly in whole blood to avoid issues with data interpretation related to cell isolation procedures.

Results: Blood samples from Alzheimer's diseases patients with dementia revealed neutrophil hyperactivation associated with increased reactive oxygen species production and increased levels of intravascular neutrophil extravascular traps. The homeostasis of circulating neutrophils in these patients also changed: the ratio between the harmful hyper-reactive CXCR4 high/CD62L low senescent and the CD16 bright/CD62L dim immunosuppressive neutrophil subsets rose in the later stage of the disease. Interestingly, these abnormalities were greater in fast-decliner than in slow-decliner patients.

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P070-T | Developmental changes of the multi-unit activity evoked by the single whisker stimulation in the neonatal rat

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The barrel system is extremely important for rodents. Using whiskers the rodents receive the major part of the sensory information about the external world. The striking feature of neurons in the barrel system is that they respond differently depending on the angular speed and direction of the displaced whisker. While high neuronal sensitivity for whisker displacement was shown in adult barrel system, little is known about the emergence of the velocity sensitivity during development. Here we exploit the evoked cortical multi-unit activity (MUA) to investigate the changes in the evoked cortical response during the critical period of barrel system development (the first postnatal week). Using a combination of the optical intrinsic signal (OIS) and electrophysiological recordings we explored the quantitative parameters of the MUA in the single barrel, received the sensory input from the deflected whisker. Optimized OIS imaging was used to detect the localization of the principal barrel, followed by the positioning of the multisite silicon probe, that allowed us to record electrophysiological activity simultaneously at different cortical depths of one cortical column. We found that during the first postnatal week, there is a progressive increase in the number of the MUA in the granular layer evoked by the whisker shift at the same deflection angle (regression coefficient is 0.96 ± 0.07 , $n = 3$, P4-7 rat pups). Variation of the angular speed of deflection didn't affect the developmental phenomena and stronger MUA response was seen in older animals. We suggest that developmental increase of the MUA is associated with the maturation of the whisker-to-barrel pathway and also contributes to the development of the angular tuning and velocity sensitivity in the neonatal barrel system during the critical period of development. The work was supported by RSF grant 16-15-10174 and performed in the frame of the Program of competitive growth of Kazan Federal University.

P071-T | Effect of Nutlin-3a, RG-7112, Carfilzomib and MG-132 on accumulation of p53 in eukaryotic HEK293 cell line

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Tumor suppressor p53 is a well-known transcription factor for multiple genes regulating cell cycle and apoptosis. MDM2 is its negative regulator that binds the p53 transactivation domain and inhibits the ability to activate transcription. MDM2 acts as an E3 ubiquitin ligase that targets p53 for 26S proteasomal degradation. Overexpression of MDM2 is one of the main reasons for impaired functions of the wild type p53. Therefore, it is important to better understand the effect of MDM2 and proteasome inhibitors on molecular and cellular processes in eukaryotic cells. In the reported work we aimed to investigate the effect of p53 activation in HEK293FT cell line using selective MDM2 inhibitors (Nutlin-3a and RG-7112) and 26S proteasome inhibitors (Carfilzomib and MG-132). A range of molecular biology methods were applied including mammalian cell transfection, gel electrophoresis and immunoblotting. The effect of MG-132, Carfilzomib, Nutlin-3a and RG-7112 on the p53 level in HEK293FT cell line was assessed for incubation periods of 24 and 48 hours. We observed that higher inhibitor concentrations lead to a more substantial increase in cellular p53 levels. Particularly, the effect of Carfilzomib and Nutlin-3a on accumulation of p53 was stronger than that of RG-7112 and MG-132. Overall, our results might contribute to the development of novel therapeutics for regulation of p53-mediated processes. The study was supported by Program of Competitive Growth of Kazan Federal University. Immunoblotting experiments were funded by RFBR research grant 16-34-60213 mol_a_dk.

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