T11-088C

Microglial Rac1 is essential for microglia-synapse crosstalk and cognitive performance

T. O. Almeida[1,2,3], R. Socodato[1,2], C. C. Portugal[1,2], E. C.S. Santos[1,4], J. Tedim-Moreira[1,4], J. Galvão-Ferreira[1,4], T. Canedo[1], A. Magalhães[1,3], T. Summavielle[1,2,5], J. B. Relvas[1,2,4]

[1] *Instituto de Investigação e Inovação em Saúde (i3s), Universidade do Porto, Porto, Portugal*
[2] *Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal*
[3] *Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*
[4] *Faculdade de Medicina, Universidade do Porto, Porto, Portugal*
[5] *Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal*

Microglia, the main immune defenders of the brain, rapidly detect and react to stimuli due to constant extension and retraction of their processes. When engaged by external cues, that can be either inflammatory or products resulting from synaptic activity, microglia dramatically change their morphology and initiate a response to reestablish brain homeostasis. Additionally, microglia can also regulate and sustain synaptic activity by secreting a plethora of factors. While some of these factors are already described, there is still much to understand on how exactly microglia-secreted factors modulate synaptic function. Rac1, a well-known member of the Rho GTPase family, is a critical regulator of actin cytoskeleton dynamics and reorganization. Furthermore, Rac1 is a component of NADPH oxidase complex, a key element for phagocytic cup formation and it also regulates NF-κB pathway activation. In the central nervous system (CNS), Rac1 is involved in axon guidance and growth, but it is also linked with Alzheimer´s disease, since it regulates the expression of amyloid precursor protein in hippocampal neurons. Although extensively studied in other cell types in and outside of the CNS, there is a profound knowledge gap on how Rac1 regulates microglia function in homeostasis and in response to external stimuli.

Combining cell-specific conditional gene ablation, RNAseq, flow cytometry, immunofluorescence, proteomics and phosphoproteomics, FRET live cell imaging and mouse behavior, we aimed at describing for the first time Rac1 roles in microglial function. We observed that microglia specific Rac1 ablation impaired the capacity of microglia to sense and respond to changes in their local environment. To promote changes on the brain environment, we performed a protocol of environmental enrichment (EE), mimicking currently used therapeutic approaches for enhancing brain plasticity in patients with brain disorders. EE had a profound impact on the microglial phosphoproteomic landscape, showing a strong effect on Rho GTPase signaling. Interestingly, we showed that Rac1 signaling was the most significantly altered pathway, followed by Cdc42 and RhoA signaling, allowing us to define a hierarchy between them. Besides, EE led to an overall improvement of cognitive performance. Strikingly, ablating Rac1 from microglia completely prevented this EE-dependent cognitive enhancement and disrupted microglia-synapse crosstalk, ultimately impacting the synaptic proteomic and phosphoproteomic profiles of these mice.Overall, this is a first step into understanding how Rac1 mediates microglial responses to their local environment. This places Rac1 as an essential target for further studies and reinforces the importance of Rho GTPases signaling for adult microglial function.

Acknowledgement

This work was funded by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., in the framework of the project PTDC/MED-NEU/1677/2021.

CCP and RS hold employment contracts financed by national funds through FCT—in the context of the program-

contract described in paragraphs 4, 5, and 6 of art. 23 of Law no. 57/ 2016, of August 29, as amended by Law no. 57/2017 of July 2019
This work was financed by the FSE - Fundo Social Europeu - through Norte2020 - Programa Operacional Regional Norte and by Portuguese funds through FCT - Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior. FCT scholarship reference: SFRH/BD/147981/2019.
TA and RS equally contributed to this work.