ROLE OF THE NLRP3 INFLAMASSOME IN THE PATHOPHYSIOLOGY OF BIPOLAR DISORDER

A.C. Pereira¹, I. Ferreira¹, B.M. Neves², S. Morais^{3,4}, N. Madeira^{3,4}, V. Santos^{3,4}, A. Macedo^{3,4}, A. Carmo⁵, F. Rodrigues^{5,6}, M.T. Cruz^{1,7}, C. Pereira^{1,8}

¹University of Coimbra, Center for Neuroscience and Cellular Biology CNC, Coimbra, Portugal; ²University of Aveiro, Department of Medical Sciences and Institute for Biomedicine (iBiMED), Aveiro, Portugal; ³Centro Hospitalar e Universitário de Coimbra (CHUC), Department of Psychiatry, Coimbra, Portugal; ⁴Faculty of Medicine-University of Coimbra, Institute of Psychological Medicine, Coimbra, Portugal; ⁵Centro Hospitalar e Universitário de Coimbra (CHUC), Department of Clinical Pathology, Coimbra, Portugal; ⁶University of Coimbra, Faculty of Medicine, Coimbra, Portugal; ⁷University of Coimbra, Faculty of Pharmacy, Coimbra, Portugal; ⁸Faculty of Medicine-University of Coimbra, Institute of Biochemistry, Coimbra, Portugal.

INTRODUCTION

Bipolar disorder (BD) is a mental illness characterized by mood swings between depression and mania resulting in cognitive and functional impairments that require lifetime treatment. Few treatments are currently available with limited efficacy and delayed/misdiagnosis is frequent due to biomarkers absence. Alterations of cellular resilience, endoplasmic reticulum (ER) stress response, mitochondrial function and innate immunity have been implicated in BD pathophysiology. Aforementioned events are closely associated with ER-mitochondria contacts, the so-called Mitochondria-Associated Membranes (MAMs). These lipid raft-like domains allow physical and functional communication between ER and mitochondria, which is crucial for inter-organelle Ca²⁺ transmission controlling mitochondrial bioenergetics and pro-survival/pro-death pathways and determining cell fate under stressful conditions. Alterations in MAMs have been described in several pathological conditions.

Aim: To unveil in human monocytes the link between ER stress and innate immunity disturbances in BD pathophysiology and to disclose the role of MAMs on ER stress-induced sterile inflammation.

RESULTS

The ER stress response is impaired and the NLRP3 inflammasome is activated in BD primary monocytes

Figure 1 – (A) Human monocytes derived from healthy subjects and BD patients (4-5 males per group, 22-37 years old) were plated in a 12 multiwell plate (0.8x10⁶/mL) and exposed to tunicamycin (TM 10 µg/mL) for 8 hours. Induction of the PERK UPR signaling pathway was evaluated by measuring the levels of ATF4 by Western blotting in total cell extracts. β-Tubulin I was used as a protein loading control. (B) Human monocytes derived from healthy subjects and BD patients (4-5 males per group, 22-37 years old) were plated in a 96 multiwell plate (0.8x10⁶/mL) and exposed to tunicamycin (TM 10 µg/mL) for 8 or 32 hours. As a measure of NLRP3 activation, IL-1β levels in cell culture supernatants were quantified using an ELISA kit. Statistical analysis: One-way ANOVA with Dunnett's post-hoc test (*p<0.05; compared to basal conditions).

MAMs components are affected by ER stress in BD monocytes

Figure 2 – Human monocytes derived from healthy subjects and BD patients (4-5 males per group, 22-37 years old) were plated in a 12 multiwell plate (0.8x10⁶/mL) and exposed to tunicamycin (TM 10 µg/mL) for 8 hours. Levels of Mitofusin 2 and Sigma-1R were evaluated by Western blotting of total cell extracts. β-Tubulin I was used as a protein loading control. Statistical analysis: One-way ANOVA with Dunnett's post-hoc test (**p<0.01; compared to basal conditions).

Inflammatory status in BD patients

Clinical parameters	BD patients	Control	p value
Age (years)	27.80 ± 2.20	28.20 ± 2.48	0.91
Leukocytes (x10 ⁹ /L)	8.04 ± 1.37	6.72 ± 0.34	0.38
Neutrophils (x10 ⁹ /L)	4.92 ± 1.05	3.88 ± 0.35	0.37
Lymphocytes (x10 ⁹ /L)	2.28 ± 0.31	1.94 ± 0.36	0.49
Monocytes (x10 ⁹ /L)	0.68 ± 0.08	0.52 ± 0.05	0.13
Eosinophils (x10 ⁹ /L)	0.10 ± 0.04	0.30 ± 0.09	0.08
Basophils (x10 ⁹ /L)	0.02 ± 0.02	0.04 ± 0.02	0.54
Platelets (x10 ⁹ /L)	248.60 ± 24.10	197 ± 19.71	0.14
Hemoglobin (g/dL)	15.14 ± 0.26	15.42 ± 0.25	0.46
Ferritin (ng/mL)	215.60 ± 55.44	171.40 ± 61.64	0.60

Table 1. Biochemical parameters analyzed on healthy individuals versus BD patients (5 males per group, 22-37 years, mean values ± SEM). Statistical analysis between both groups was determined using the *t*-test.

ER stress activates the NLRP3 inflammasome in THP-1 monocytes

Figure 3 – (A) THP-1 cells were plated in a 6 multiwell plate (0.8x10⁶/mL) and exposed to tunicamycin (TM 5 or 10 µg/mL), during the indicated time periods (1-24 hours). PERK UPR activation was evaluated by Western blotting of total cell extracts by measuring ATF4 levels. β-Tubulin I was used as a protein loading control. Statistical analysis: One-way ANOVA with Dunnett's post-hoc test (****p<0.0001 compared to Ctrl). (B) THP-1 monocytes were plated in a 12 multiwell plate (0.8x10⁶/mL) and exposed to tunicamycin (TM 5 or 10 µg/mL) for 8 hours, LPS (1 µg/mL) for 24 hours and ATP 5 mM for 30 minutes. As a measure of NLRP3 activation, IL-1β levels in cell culture supernatants were quantified using an ELISA kit. Statistical analysis: *t*-test was used for LPS-ATP and Ctrl (####p<0.0001), LPS+ATP and LPS-ATP (*p<0.05), LPS+vehicle and vehicle (ttttp<0.0001) comparisons, and One-way ANOVA with Dunnett's post-hoc test (*p<0.05, compared to LPS+vehicle).

MAMs are enhanced by ER stress in THP-1 monocytes

ER stress does not affect ROS production but depolarizes the mitochondrial membrane in THP-1 monocytes

Figure 5 – (A) THP-1 cell line was plated in a 12 multiwell plate (0.8x10⁶/mL) and exposed to tunicamycin (TM 5 or 10 µg/mL) for 8 hours. Production of mitochondrial superoxide was measured using the fluorescence probe MitoSOX. (B) Human THP-1 monocytes were plated in a 48 multiwell plate (1x10⁶/mL) and exposed to tunicamycin (TM 5 or 10 µg/mL) for 8 hours. Alterations in mitochondrial membrane potential due to TM exposure were determined using the TMRE fluorescent probe. FCCP (50 µM) was used as a positive control. Statistical analysis: One-way ANOVA with Dunnett's post-hoc test (*p<0.05, **p<0.01 compared to vehicle).

CONCLUSION

In BD patients-derived monocytes:

- ER stress induces NLRP3 inflammasome activation;
- MAMs' composition is affected under ER stress conditions;
- inflammatory markers are increased in comparison with controls.

In human THP-1 monocytes:

- ER stress induces NLRP3 activation independently of mitochondrial ROS production and impairs ER-mitochondria contacts at MAMs affecting mitochondria function.

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