

IAF/IAA SPACE LIFE SCIENCES SYMPOSIUM (A1)
Biology in Space (8)

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EPIGENETIC EVENTS IN MICE' OVARIES AFTER MODELLING MICROGRAVITY

Abstract

Weightlessness has a negative impact on various systems of the human body, including on the cardiovascular, muscle and skeletal systems, which can impede the deep space exploration. But, for reproductive system there were very limited data. Therefore, the aim of this work was to estimate the total DNA methylation level, the content of the intermediate product 5-hydroxymethylcytosine (5hmC) and the enzymes controlling these processes (DNA methylases DNMT1, DNMT3a, TET demethylases and HDAC1 deacetylase) in the ovaries of mice after long-term antiorthostatic suspension. Microgravity effects were simulated using the Ilyin-Novikov standard model of antiorthostatic suspension modified by Morey-Holton during 23 days. The animals were randomly divided into two groups: C (n=7), control group; HS (n=7), the 23-day suspension group. Total DNA was isolated from the frozen tissues by a phenol/chloroform method. The total methylation level was estimated through restriction analysis (Thermo Scientific, USA), the content of 5hmC – by dot-blotting with specific antibodies and proteins content – by Western blot with specific antibodies (all primary antibodies – Abcam, UK). The total methylation level in the ovaries was reduced after 23 days of hindlimb suspension by 20%. The content of S-phase DNA methylase DNMT1 and de novo methylase DNMT3a did not change after antiorthostatic suspension. But the relative content of the active demethylase TET2 increased after suspension by 16%. Content of histone acetylase HAT1 did not change, but the deacetylase HDAC1 content decreased after disuse by 17%. The obtained results indicate that in the ovarian cells of mice, after a 23-day antiorthostatic suspension, a hypomethylated state was established, in the absence of changes in the content of 5hmC. However, Western blot data indicate that the content of DNMT1 and DNMT3a methylases did not change, while the TET2 demethylase content increased. In this case, it can be assumed that the establishment of a hypomethylated state is due to active complete demethylation of the target sites, without accumulation of an intermediate 5hmC product. This work was financially supported by the program for fundamental research SSC RF – IBMP RAS; program “Cell and Molecular Biology” of the RAS Presidium; Russian Academic Excellence Project 5-100.