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Abstract

Microgravity Exposure Alterations of Cellular Junctions Proteins in TCam-2 Cells: Localization and Interaction †

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Abstract: One of the most important hazards of the space environment is microgravity, which causes an alteration in the physiology of different systems, including the reproductive one. It is widely accepted that cytoskeleton is the microgravity-sensitive apparatus of the cells, and that cytoskeletal modifications are responsible for microgravity-triggered cell alterations. We established a 3D free-floating culture system from TCam-2 cell, a human seminoma cell line, and then exposed the obtained TCam-2 spheroids for 24 h at unitary gravity (UG), or under a simulated microgravity condition (SM), using the random position machine (RPM). We tested the cytoskeletal and junctional features of these samples using Western blot and confocal microscopy analysis to elucidate the impact of microgravity on the adherent and occluding junctions of TCam-2 spheroids. The junctional ultrastructure was studied using transmission electron microscopy (TEM). TEM analysis revealed the presence of occluding junctions both in UG or SM samples. Even if Western blot revealed no quantitative difference in actin and occludin proteins both in UG and SM exposed samples, fluorescence colocalization analysis showed a significative increase in the colocalization area of occludin and actin proteins in the superficial layer of TCam-2 spheroids grown in RPM conditions. This result let us speculate that tight junction functionality is different in UG and SM exposed spheroids. As far as adherent junctions are concerned, TEM analysis revealed adherent junctions both in UG or SM samples. Moreover, we observed by Western blot a trend in terms of the increase in the vimentin expression in SM exposed spheroids. Confocal microscopy analyses confirmed this significant increase. All together, these data suggest that simulated microgravity conditions in TCam-2 spheroids alter the tight junction assembly, while the increase in the intermediate filament's structures can in part be associated with an enrichment in the adherent junctions. A functional investigation is needed to more deeply clarify this hypothesis.

Keywords: microgravity; cytoskeleton; TCam-2 cell

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