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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 space environment is microgravity, which causes 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 of microgravity-triggered cell alterations. We established a 3D free floating culture system from TCam2 cell, a human seminoma cell line, and then exposed the obtained TCam2 spheroids for 24h at unitary gravity (UG) or under simulated microgravity condition (SM), by using the Random Position Machine (RPM). We tested cytoskeletal and junctional features of these samples by western blot and confocal microscopy analysis to elucidate the impact of microgravity on adherent and occluding junctions of TCam2 spheroids. The junctional ultrastructure was studied by 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 of actin and occludin proteins both in UG and SM exposed samples, fluorescence colocalization analysis showed a significative increase of colocalization area of occludin and actin proteins in the superficial layer of TCam2 spheroids grown in RPM conditions. This result let us speculate that tight junction functionality is different id UG and SM exposed spheroids.

As far as adherent junctions is concerned, TEM analysis revealed adherent junctions both in UG or SM samples. Moreover, we observed by western blot a trend in increase of vimentin expression in SM exposed spheroids. Confocal microscopy analyses confirm this significant increase..

All together these data suggest that simulated microgravity conditions in TCam2 spheroids alters tight junction assembly, while the increase in intermediate filaments structures can in part be associated with an enrichment in adherent junctions. Functional investigation are needed to deeper clarify this hypothesis.

Keywords: Microgravity; Cytoskeleton; TCam2 cell