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Culture cells in a model of microgravity

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Abstract

The main objective of the work was to clarify the question – how will cell cultures functional state change after microgravity simulation when the shift in full strength direction takes place? Proliferation processes and apoptosis intensity in cell lines of rat glioma and human fibroblasts were compared in changing the position of flasks with cell culture in relation to the horizon. The detection of apoptosis and necrosis processes was carried out using flow cytofluorimetry. It was found that the change in full strength direction provides an inhibitory effect on tumor glial cells and fibroblasts' proliferative activity enhances along with inhibition of apoptotic processes. Intensification of apoptotic processes in glioma cells and attenuation of cell death processes in normal cells – fibroblasts – are the result of cell cooperation disturbance.

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Introduction

To assess the peculiarities and perform comparative analysis of viability, phenotypic characteristics, proliferative activity of linear cell cultures (C6 rat glioma cells and A549 human lung carcinoma, FLv-line human fibroblasts) after the modeling of microgravity effects.

Material and Methods

Cell lines of C6 rat glioma and FLv-line human fibroblasts were obtained from the Russian Cell Culture Collection of Vertebrates (Cytology Institute of Russian Academy of Sciences, Saint-Petersburg).

C6 rat glioma cells and FLv human fibroblasts were cultivated (concentration 2×10^5 cells/ml) in 25 ml flasks in F10 medium with 10% fetal bovine serum and 10^{-4} g/ml gentamycin sulfates. Flasks were placed in CO_2 incubator at 5% CO_2 and 37°C. Microgravity was modeled using UN-KTM2 clinostat for 4 hours at 10 rpm (1).

Cell viability was monitored while using a clinostat for 4 hours. The results of the experiments showed that the most pronounced changes in cells happened in 24 hours (2) after the change of flasks' position relative to the horizon. So flasks' position was changed to angle 60° from the horizontal position during experiments with C6 rat glioma cells and human fibroblasts cultures. Rotation was carried out in 40-48 hours after reaching 70% confluence.

Annexin V (AnV) – propidium iodide (PI) (Annexin V-FITC Apoptosis Detection Kit, No 556547, lot No 2195781, BD Pharmingen™, USA) system was used for detection of apoptosis with the help of flow cytofluorimetry. The distribution of dyes in cells allow establishing their characteristics and dividing them into living, necrotic and apoptotic.

Results are expressed as mean ± standard error of mean. Differences between means were evaluated by one-way analysis of variance (ANOVA) or Student's test for unpaired observations.

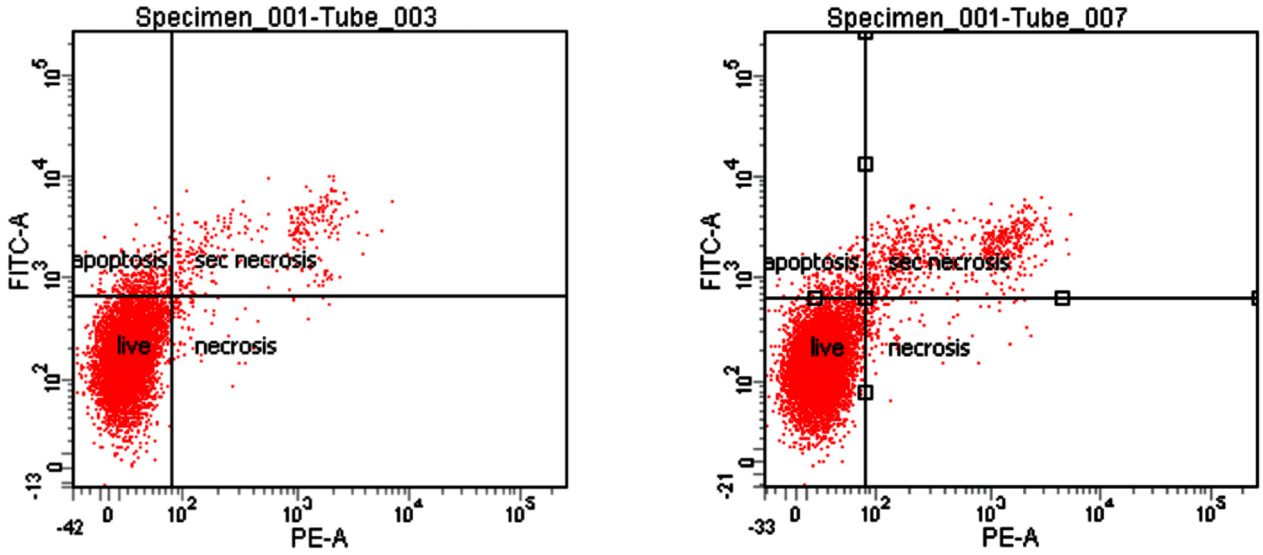

Figure 1. Distribution of the living, apoptotic and necrotic cells of rat C6 glioma in the analysis of the content of annexin V flow cytometer: on the left - in the vials, which over the entire experiment was in a horizontal position, on the right - after turning the vials to 60 °.

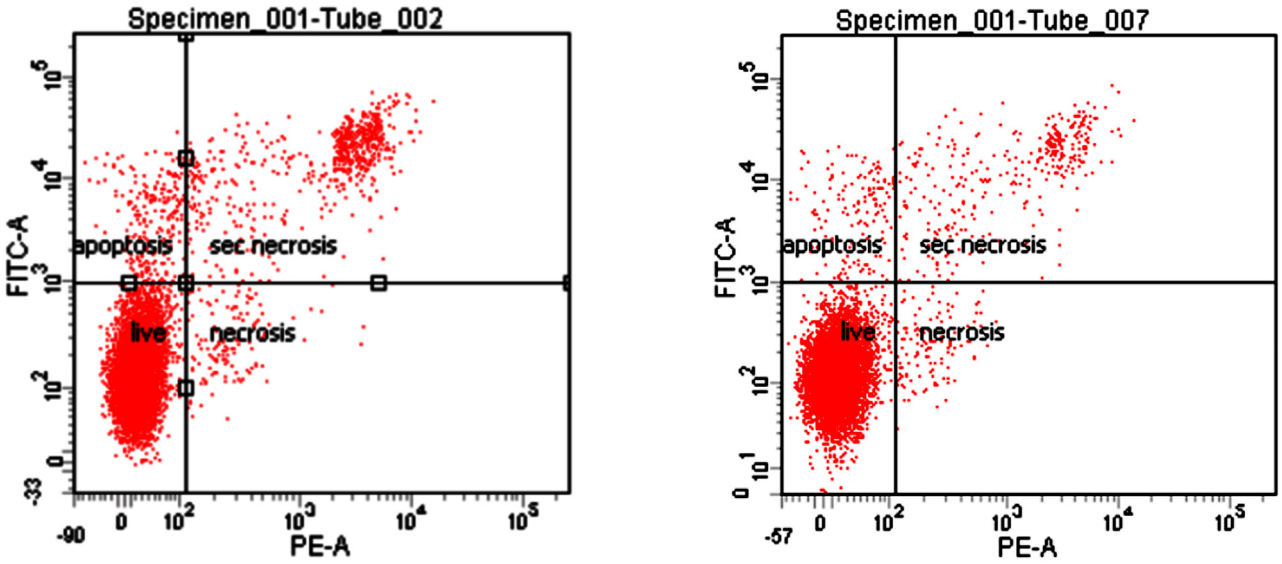

Figure 2. Distribution of the living, apoptotic and necrotic cells of human fibroblasts Flv in the analysis of the content of annexin V flow cytometer: on the left - in the vials which over the entire experiment was in a horizontal position, on the right - after turning the vials to 60 °.

Results and Discussion

Viability reduction of C6 rat glioma cells was demonstrated *in vitro* experiments after the change of full strength direction (the modeling of microgravity effects). The increase of necrotic cells number was observed in C6 rat glioma. The next findings were obtained in fibroblasts culture in simulated microgravity: the enhancement of the processes of proliferation, adhesion molecules expression (cadherin, β2-integrin), cloning efficiency, IL-6 level in culture medium with FLv human fibroblasts and the formation of multilayer cell populations in 3D ceramic structures. he shift of full strength direction (the modeling of microgravity effects) was shown for the first time to be one of the factors regulating the processes of proliferation and death of normal and pathological cells.

References

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