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# A LONG-TERM 10G-HYPERGRAVITY EXPOSURE PROMOTES CELL-CELL CONTACTS AND REDUCES ADHESIVENESS TO A SUBSTRATE, MIGRATION, AND INVASIVENESS OF MCF-7 HUMAN BREAST CANCER CELLS

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***Background*: G-force is a fundamental force controlling human cells. Cancer is one of the 4 major health challenges in the Space missions. Cancer in Space project evaluates the reaction of human cancer cells to the conditions of the space flights, including an exposure to high g-forces. *Aim*: Explore an impact of 10 g force on the oncogenic properties of human breast adenocarcinoma cells MCF-7. *Materials and Methods*: Cells were exposed to 10 g force for 10 days, as part of a 6-week simulation of conditions of a space flight. Then the cells were cultured for one week under normal culture conditions, before performing tests. Cell proliferation, cell viability, cell-cell contact inhibition, migration, and invasiveness were measured. Immunoblotting was used to evaluate expression of proteins. *Results*: Proliferation, cell-cell interaction and formation of 3D structures, migration, and invasiveness of cells exposed to 10 g were compared to parental cells cultured at 1 g condition. 10 g exposed cells showed a higher propensity for cell-cell contact inhibitions and lower for 3-dimensional growth in dense culture. This correlated with the decrease of proliferation in a dense culture as compared to the parental cells. The decrease of migration, adherence to a surface, and invasiveness was observed for cells subjected to the hypergravity, as compared to the parental MCF-7 cells. Enhanced expression of E-cadherin and phosphorylated pY576-FAK were observed in 10 g exposed cells but no impact on the expression of Erk, pErk, FAK and p53 was detected. *Conclusion*: The prolonged exposure of MCF-7 cells to 10 g force targets cell-cell and cell-substrate interactions.**

***Key Words*: g-force, hypergravity, human cancer cells, MCF-7, cell-cell interaction, cell-substrate interaction.**

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G-force is one of the fundamental forces controlling cellular physiology. Definition of the weight and masses of cells and cellular components defines regulatory mechanisms in cells [1–4]. Directionality and the strength of g-force impacts the cell growth, death, and migration of human cells [1, 4]. Space research has focused on studies of effects of the weightlessness defined as microgravity. The impact of high gravity force on human cells is much less explored.

Studies of the biological impact of gravity are crucial for two reasons. The first is related to the exposure of humans to high g forces in the space or on Earth, e.g., during flights. The second reason is a study of fundamental mechanisms controlling human cells in normal and disease conditions. Gravity is a fundamental force that has been without significant fluctuations during the whole history of life on Earth. The role of the gravity force is crucial in the definition of living systems, e.g., the size of organisms, biochemical processes and physiology [1–4].

Gravity-sensing mechanisms have been studied in human, animal, and plant cells (reviewed in Takahashi *et al*. [4]). On the cellular level, response to gravity includes modulation of the cytoskeleton, cell-cell and cell-substrate interactions, and on the intracellular level a number of regulatory pathways were identified [5–10]. Changes in cellular adhesion and cytoskeleton are among the most frequently observed effects of gravity, e.g., modulation of vessel formation by endothelial cells [11, 12]. Promotion of myoblasts differentiation [13], neuron-like differentiation of PC12 cells [14], and decreased count of $CD4^{+}CD8^{+}$ thymocytes [15] were observed upon exposure to hypergravity. Among response mechanisms to hypergravity, there were reported cAMP-reactive proteins [16], and c-fos, ROCK/Rho-GTP, and the PI3K signaling [17]. Enhanced release of reactive oxygen species upon exposure to hypergravity was also reported [18]. Omics studies, e.g., sequencing data, showed that 15 min of hypergravity induced expression of a significant number of genes, e.g., ATPase subunits and the cluster of differentiation molecules [19]. However, most of the altered gene expression was transient [19, 20]. This suggests that a longer exposure to hypergravity has to be studied to detect permanently acquired changes.

A number of studies showed that human cancer cells react to hypergravity in a way that affects their oncogenic properties. Stress signaling response, inhibition of cell proliferation, and modified patterns of cell-cell interaction and migration were observed [5–8, 21, 22]. However, the available data

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*Abbreviations used*: CBB – coomassie brilliant blue; FAK – focal adhesion kinase.

are scarce and fragmentary. Here we report results obtained with MCF-7 cells. We showed that the exposure of MCF-7 human cancer cells to 10 g force for 10 days enhanced cell-cell contacts, and decreased formation of 3D structures by the cells, cell-substrate attachment, migration, and invasiveness, as compared to the parental cells. This is accompanied by enhanced expression of E-cadherin and phosphorylated focal adhesion kinase (pFAK).

## MATERIAL AND METHODS

***Cells and reagents.*** Cells were obtained from ATCC and were in culture for less than a year since the receiving. MCF-7 (HTB-22, human breast adenocarcinoma cells) cell line was used in this study. Supplementary data (Supplementary Figures S1, S2, S3 and S4) contain results obtained with MDA-MB-231 (HTB-26, human breast adenocarcinoma cells), 786-0 (CRL-1932, human kidney adenocarcinoma cells), and ACHN (CRL-1611, human kidney adenocarcinoma cells).

***Protocol of cell exposure to 10 g force.*** Four types of culture were used. The first type of culture, i.e., parental cells, were kept in a standard culture in plastic flasks (cells are annotated as "parental"). For three other conditions, cells were cultured in 2-ml long-term storage cryotubes (Thermo Scientific, Nalgene USA), (10,000 cells per 2 ml of complete DMEM medium) (Gibco, Life Technologies, USA) with 10% FBS (Gibco, Life Technologies, USA) and 1% penicillin/streptomycin (5000 U/ml, Gibco, Life Technologies, USA). The culture conditions were optimized to ensure the survival of cells for 6 weeks of culture. The 6-week period was selected to simulate the culture conditions to which the cells would be exposed during the space flight, e.g., to ensure sufficient supply of nutrients and oxygen. For the second condition, cells were cultured at 37 $^0$C in a centrifuge at 10 g force for 10 days continuously (cells are annotated as "10g"). The exposure to 10 g force was performed throughout 6 weeks of culture in cryotubes. For the third condition, cells were cultured in a vertical circular rotator that mimicked different positions of the tubes toward 1 g gravitational force (cells are annotated as "rotation"). For the fourth condition, cells were cultured in tubes without rotation or 10 g force; in cryotubes cells could not attach to the substrate (cells are annotated as "37C"). After culturing in cryotubes for 6 weeks, the cells of all 4 conditions of experimental settings were recovered into standard plastic flasks, cultured for additional 1 week, and then were tested as described for each type of tests. Nine 6-week simulation experiments were performed, with 2 independent experiments comprising generation of 10 g exposed cells during the 6-week simulation.

***Cell proliferation assay (by cell number detection).*** Cells were seeded in 6-well plates, and cultured for 48 or 72 h. After incubation, cells in the plates were fixed with 70% ethanol, 100% ethanol, air-dried and stained with coomassie brilliant blue (CBB). Cells would be stained in blue color, and the intensity of the staining reflects number of cells in a well. Staining of cells by CBB shows a linear correlation between intensity of the staining and number of cells [23]. Quantification of the staining intensity was performed by scanning of images of the wells, followed by quantification of stained areas with the use of the ImageJ software (https://imagej.nih.gov/ij/).

***Cell-cell contact inhibition and formation of 3D structures assay.*** Cells were seeded in standard 6-well plates (Thermo Scientific Nunc cell-culture treated multi-well plates with the Nunclon Delta surface treatment, supplied by MLM, Doha, Qatar) and cultured for 3 weeks. After incubation, cells in the plates were fixed with 10% acetic acid and 10% methanol in the water, air-dried and stained with CBB. Cells in stained plates were evaluated under a microscope, and images were taken. The images were processed to generate 3D images with the IMAGEtoSTL conversion tool that is available at https://imagetostl.com/. The images were used for quantification by calculation of the ratio of the cell culture surface areas of cellular overgrowth and formation of 3D structures in relation to the total cell culture area.

***Cell invasiveness (membrane invasiveness) assay.*** Corning Matrigel Invasion Chamber 24-well plate was used (DLW354480, Sigma Aldrich/Merk KGaA, Darmstadt, Germany). Packages were removed from –20 °C and kept at room temperature for 5 min, 500 µl of complete DMEM media with 10% FBS and 1% penicillin/streptomycin were added to the lower well and the upper inserts, the plates were allowed to rehydrate at 37 °C and 5% $CO_2$ for 2 h. After incubation, media were removed from the plates, 750 µl of complete DMEM media was added to the lower well, upper inserts were replaced in the wells, 500 µl of cells were seeded with 50,000 cells/well. Four replicates of each cell condition (parental, rotation, and 10 g exposed) were seeded in each plate. Plates were incubated at 37 °C and 5% $CO_2$ for 24 h. After incubation, media were removed from the inserts, cells were fixed with 70% ethanol for 10 min, then stained with 0.5% crystal violet in 20% methanol for 10 min. Non-invading cells were wiped gently using a cotton swab. Inserts were washed with distilled water and air-dried. Images were taken under ×10 and ×20 magnifications.

***Cell migration (membrane migration) assay.*** Cells were seeded on the top of the ChemoTx® System 96-Well cell migration membrane (NeuroProbe, Gaithersburg, USA), 300 µl of complete DMEM medium was added to the lower chamber, 30 µl of cells were seeded on the top of the membrane with a 50,000 cells/well. Four replicates of each cell condition (parental, rotation, and 10 g) were seeded in each plate. Membranes were incubated at 37 °C and 5% $CO_2$ for 24 h. After incubation, cells on the top of the membrane were fixed with 70% ethanol for 10 min, then stained with 0.5% crystal violet in 20% methanol for 10 min. Non-migrated cells were wiped gently using a cotton swab, membranes were washed with distilled water

and air-dried. Images were taken under ×10 and ×20 magnifications.

***Cell-substrate adhesion assay.*** Cells were seeded in 12-well plates with 50,000 cells/well, cells were incubated for 1, 2, 4, and 24 h in a humidified incubator at 37 °C and 5% $CO_2$. The standard Thermo Scientific Nunc cell-culture treated multi-well plates with the Nunclon Delta surface treatment were obtained from Nunc/Thermo Fisher Scientific (supplied by MLM, Doha, Qatar). After incubation, media were removed, cells on the plates were fixed with 10% acetic acid and 10% methanol in distilled water, then cells were washed with distilled water, and stained with CBB for visualization of attached cells. Images of the adherent cells were taken under ×10 magnification.

***Cell migration (scratch) assay.*** Cells were seeded in 6-well Nunc standard plates until the cells reached a dense monolayer. Scratches were made in the cellular monolayers [24]. Images were taken immediately after the initial scratch. After 24 h, cells were fixed with 10% acetic acid and 10% methanol dissolved in distilled water and stained with CBB for visualization of cells. The closure of the scratch was calculated as a ratio of the open non-populated by cells distance to the initial scratch-distance.

***Cell proliferation and cell viability assay (by MTT).*** MTT assay measures the activity of a metabolic enzyme and reflects a number of cells [25]. If cells proliferate and are metabolically active, the MTT signal would increase. If cells are not viable and the number is decreasing, the MTT signal would be lower than the initial signal at the cells seeding. The cells were seeded in 96-well plates with 6 to 12 repeats per condition. Cells were seeded at 1,000 and 10,000 cells/well. MTT reagent (Sigma Aldrich, supplied by MLM, Doha, Qatar) was added to cells for the last 6 hrs of incubation. The generated tetraformazane crystals were dissolved in DMSO solution, and absorbance was measured at 570 nm. The MTT signal after 24 h was considered as the initial value and was set to 100%. The signals after 48 and 72 h of incubation were compared to the initial signals. The generated MTT signal was measured in a reader at 570 nm.

***Immunoblotting.*** Antibodies used in this study were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Abcam (Cambridge, UK). Antibodies were as follows: E-cadherin (sc-8426, mouse monoclonal IgG1), vimentin (sc-6260, mouse monoclonal IgG1), Focal Adhesion Kinase (FAK; sc-557, rabbit polyclonal IgG), phospho-FAK (sc-16563-R, pY576-FAK, rabbit polyclonal IgG), p53 (sc-126, mouse monoclonal IgG2a), extracellular-signal related kinase (Erk1/2; SC-514302, mouse monoclonal IgG2a), phospho-Erk (sc-7383 mouse monoclonal IgG2a), actin (ab-8227, rabbit polyclonal). Cell extracts were separated by SDS-PAGE and transferred onto membranes. After blocking with 5% BSA, membranes were probed with primary antibodies at dilutions 1:1,000, and after incubation with secondary horseradish peroxidase conjugated antibodies, the signal was detected by the chemiluminescence reaction. Chemiluminescence images were recorded in iBright instrument (Invitrogen, iBright FL1500 Imaging System).

***Statistics.*** Calculations of the significance of observed changes were performed by one-way ANOVA test. The significance threshold was set to $p < 0.05$.

## RESULTS

***10 g hypergravity promotes contact inhibition.*** We applied a long-term 10-day exposure of cells to 10 g force to induce mechanisms that would be permanently acquired by cells. These acquired features would not be leveled to the initial state after returning cells to the normal 1 g culture condition. Permanently acquired features would be present even after 10 g force would be removed. Therefore, we cultured the cells under normal conditions for 1 week after termination of 10 g exposure, before starting testing the cells.

We observed that 10 g exposed cells acquired stronger contact inhibition of growth in a dense culture. MCF-7 10 g exposed cells showed much less propensity to overgrow and formation of 3-dimensional structures on the top of cellular monolayer (Fig. 1). The calculated estimate of cell overgrowth was above 50% for the parental cells forming 3D structures and less than 20% for the 10 g exposed cells forming much smaller 3D structures (Fig. 1, 3D zoom-in panels). The calculated percent value is the ratio of the cell culture surface occupied by cellular overgrowth with 3D structures to the total cell culture surface area. Similar responses were observed also for ACHN and MDA-MB-231 cells (Supplementary Fig. S1). We noted that the exposure of cells to the constant change of the direction of the gravity force by constant rotation led to the enhancement of contact inhibition but less as compared to 10 g exposure. Therefore, exposure to 10 g force promoted cell-cell adhesion and contact inhibition of the cells as shown by the lower propensity of the 10 g exposed cells to form 3D structures.

***10 g exposure decreases cell adhesion to a substrate, migration, and invasiveness.*** The ability of cells to adhere reflects the strength of the cell-substrate interaction. The substrate we used was a standard culture plastic plate. We observed significant cell attachment in 4 h after seeding of cells (Fig. 2, *A*; Supplementary Fig. S2). Cell suspension for seeding was obtained after trypsin treatment-based detachment of cultured cells. 10 g exposed (10 g), cells cultured without substrate attachment (37C) and rotation-exposed cells showed lower adherence as compared to the parental cells. These differences were observed after 1 h and 2 h, but the number of adhered cells was not sufficient for ensuring statistical significance of the differences. After 24 h, lower adhesion was observed to 10 g and rotation-exposed cells (Fig. 2, *A*; Supplementary Fig. S2). These results show that 10 g force or the constant change of g-force directionality promotes lower adhesion of cells to the substrate. The cell-produced extracellular matrix

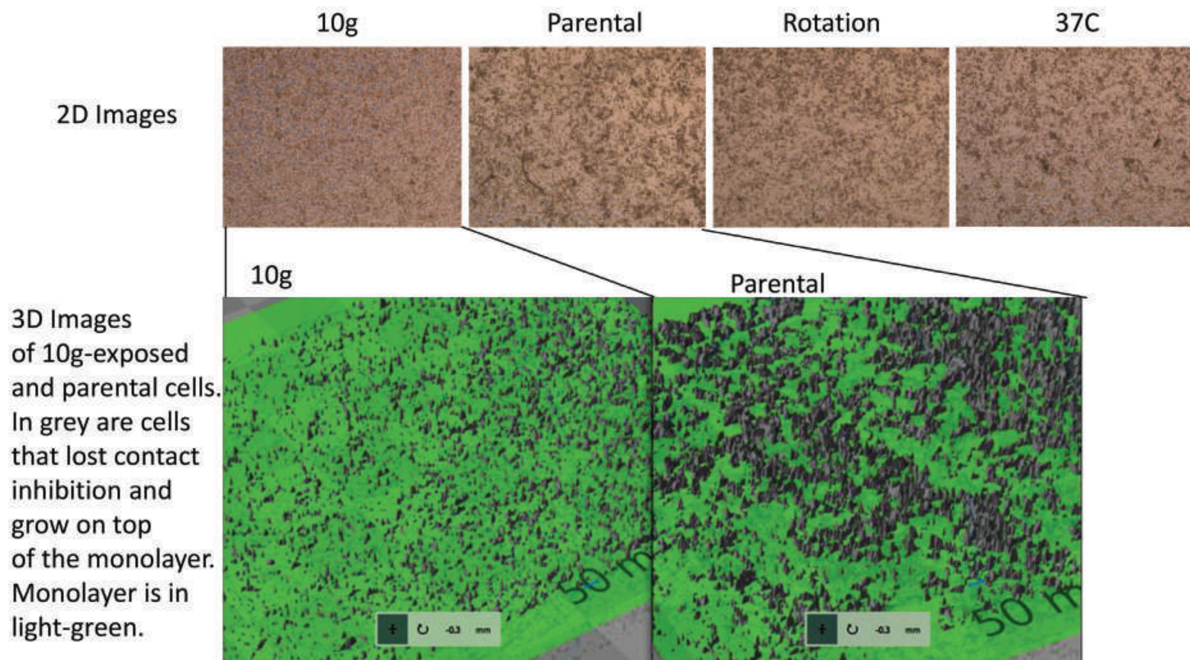


**Fig. 1.** 10 g exposure promotes cell-cell contacts leading to inhibition of formation of 3D structures by MCF-7 cells. Images show cells cultured for 3 weeks of the cell-cell contact test. Cells were treated as annotated, "10 g", 10 g exposed cells, "parental", parental cells cultured under standard conditions, "rotation", cells cultured in a rotation machine, "37C", cells cultured under anchorage-independent conditions. See description of experimental treatments in the Material and Methods section. Magnification is 10x. Lower panels show a 3-dimensional reconstruction of growing layers of 10 g exposed and parental cells. The annotated cells were cultured for 3 weeks to evaluate the cell-cell contacts. The grey color indicates cells growing on top of the monolayer indicated in green. A representative experiment out of three performed is shown. The cells for these tests were recovered as described in the Material and Methods section. Notably, the cells were subjected to a 6-week simulation culture that included 10 days exposure to 10 g force for "10g" cells, followed by a 1-week recovery under standard culturing conditions, and before performing experiments

is a strong promoter of 3D cellular growth. The observed lower adherence may be associated with the lower matrix production and with the observed lower rate of 3D structure formation (Fig. 1 and 2; Supplementary Fig. S1 and S2).

The cell-substrate interaction is crucial for the migration of cells. We observed that 10 g exposed and rotation-exposed cells showed a lower migration rate through a membrane as compared to the parental cells (Fig. 2, *B, C*). Cells that migrated through the membrane were visualized and quantified (Fig. 2, *B, C*). Quantification showed a strong inhibition of rotation-treated cells, and up to 50% inhibition for 10 g exposed cells.

The scratch assay is regarded as a fast and simple test of cell migration [23]. Our results (Fig. 2, *D*; Supplementary Fig. S3) suggested that the 10 g exposed cells migrated weaker as compared to the parental cells. Membrane migration assay allows evaluation of the cellular migration for the whole population of cells in a non-confluent culture. Scratch assay requires that cells reach a confluent. Then, after a scratch, cells bordering the open area have to be activated and begin migrating. The difference in conditions of cells subjected to the tests, e.g., confluent for scratch assay, or actively proliferating for membrane assay, may explain variations observed for 10 g and rotation-exposed cells. However, both assays showed that 10 g exposure inhibited migration of MCF-7 cells.

Invasiveness of the cells was measured by migration of the cells through the membrane pores filled with Matrigel (Fig. 3, *A, B*). In this assay, the ability to migrate through a matrix defines how many cells will reach the opposite side of the membrane. We observed that 10 g exposed cells showed lower invasiveness capacity as compared to the parental cells. This is in the agreement with the results of migration and adhesion tests. We observed that rotation-exposed cells acquired a pronounced invasive phenotype. This indicates that 10 g exposure prompted mechanisms that differ from rotation-dependent blocking of anchoring of the cells to a substrate. Thus, 10 g exposure resulted in lower rates of the cell invasiveness, migration, and adherence to a substrate.

***MTT assay showed decrease of the metabolic activity of cells in dense culture*.** MTT assay allows evaluation of cellular metabolic activity [25]. A low number of viable cells or low metabolic activity would lead to a low formation of farmazan and a low MTT signal. We observed that at the high seeding density and after 3 culture days, MCF-7 (Fig. 3, *C*) and 786-0 (Supplementary Fig. S4) 10g exposed cells showed lower MTT signal. After 3 days of culture, the cells formed high-density monolayers, and the parental cells started to form 3D structures, while 10 g exposed cells did not. This is similar to what was observed in a cell-cell contact and 3D structure formation assay. No cell death was observed, e.g.,

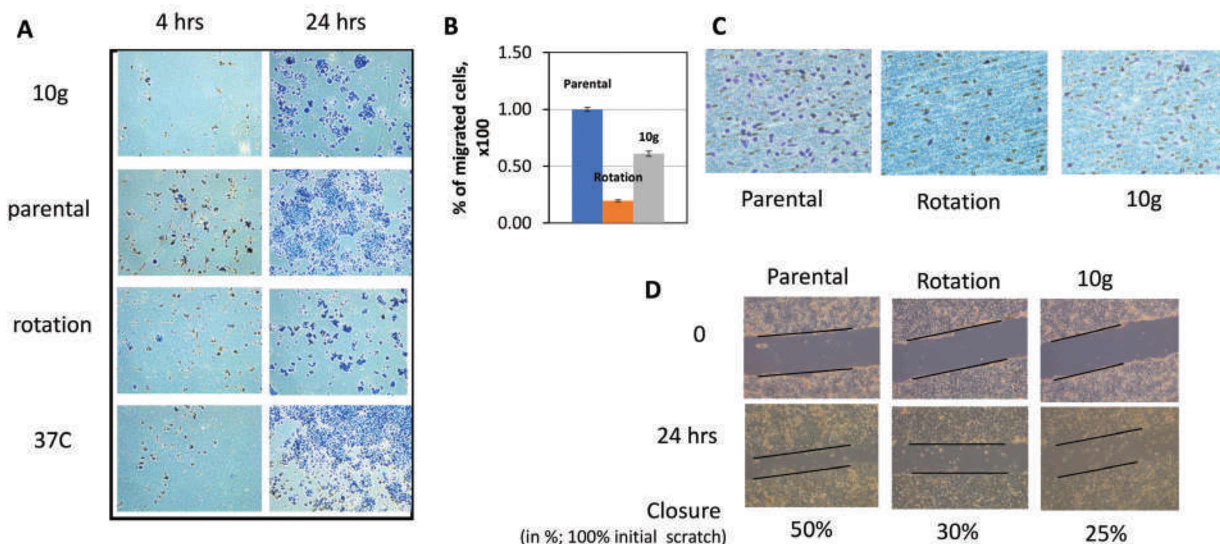


**Fig. 2.** 10 g exposure prevents cell-substrate adhesion and inhibits cell migration. A) Images show MCF-7 cells adhered to the surface after 4 h and 24 h. Adhered cells were stained (blue color). The cells were treated as annotated. Magnification is ×10. In Fig. 2, annotation of cell treatments is as in Fig. 1, and is described in the Material and Methods section. B) Graph shows quantification of cells migrated through a membrane. The ratio of migrated cells is indicated as percent with 100% (1.0 of% ×100) corresponding to the migration of parental cells. C) Images show migrated MCF-7 cells. The images are representative of the experiments that are quantified in panel (B) Magnification is ×20. D) Images of MCF-7 cells subjected to a scratch assay. Upper panels show initial scratches, with the indication of the borders of scratch. Lower panels show images of migrated cells, as a closure of the scratch after 24 h. Values of the closure of the scratch after 24 h are indicated as percent to the initial width of the initial scratch. Magnification is ×10. Representative experiments out of 4 (A, B, C) and 3 (D) performed are shown. The cells for these tests were recovered as described in the Material and Methods section

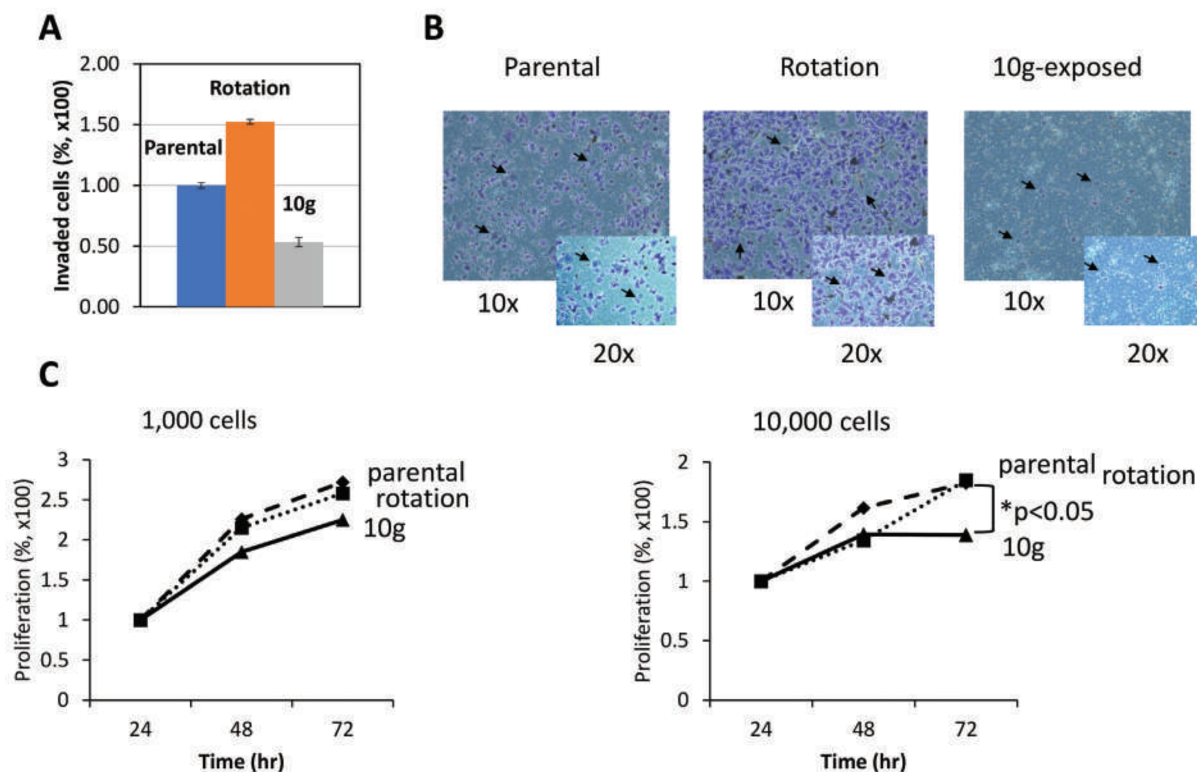


**Fig. 3.** 10 g exposure inhibits invasiveness of cells and decreases MTT signal in a dense culture. A) Graph shows the rate of invasiveness observed for parental, 10 g and rotation-exposed MCF-7 cells. Invasiveness rate for parental cells was taken as 100% (1.0). B) Images show MCF-7 cells that invaded the matrix of the membrane and migrated through the membrane. Magnifications are ×10 and ×20 as annotated for the images. C) 10 g exposure led to decrease of cell proliferation in dense culture (10,000 cells/well). MCF-7 cells were seeded at low (1,000 cells/well) and high (10,000 cells/well) concentration. Note that the cells seeded at low density (1,000 cells/well) did not reach confluent monolayer during duration of the assay. MTT assay was performed after 24, 48 and 72 h. MTT signal is shown. Annotation of cells as "parental", "rotation" and "10g" is as in Fig. 1, and is described in the Material and Methods section. Representative experiments out of 5 (A, B) and 4 (C) performed are shown. The cells for these tests were recovered as described in the Material and Methods section

no apoptotic bodies, no cellular debris. Interestingly, when cells were seeded at low density, i.e., 1,000 cells/well, the MTT signal was increasing even for 10 g exposed cells. This may be explained by the observation that the cells seeded at 1,000 cells/well did not reach a dense monolayer culture during the assay, while cells seeded at 10,000 cells/well showed a dense monolayer already after first 24 h. Inhibition of cell growth, and subsequently metabolic activity in dense culture of the 10 g exposed cells seeded at 10,000 cells/well correlates with the more than twice decrease of 3D structure formation by 10 g exposed cells as compared to parental cells (Fig. 3, *C*).

***10 g exposure enhanced expression of E-cadherin.*** Cell-cell and cell-substrate interactions are components of the oncogenic transformation of cells involved in the epithelial-to-mesenchymal transition (EMT) [26]. We observed that 10 g exposed and rotation-exposed cells had a higher level of expression of E-cadherin, as compared to the parental MCF-7 cells (Fig. 4). This correlates with the enhanced cell-cell contacts observed in the contact inhibition test (Fig. 1), i.e., when the area of the cellular monolayer free from 3D structures increased from 50% to 80%. It has to be noted that the expression of E-cadherin correlates with the strength of cell-cell contacts. Vimentin level in 10 g exposed cells did not change significantly, as compared to the parental cells. We observed vimentin isoforms of lower molecular mass. Focal adhesion kinase (FAK) is a crucial component regulating the adhesion of cells [27]. We observed that the total level of intracellular FAK did not change. However, phosphorylated pY576-FAK was elevated in 10 g and rotation-exposed cells. This correlates with increased expression of E-cadherin.

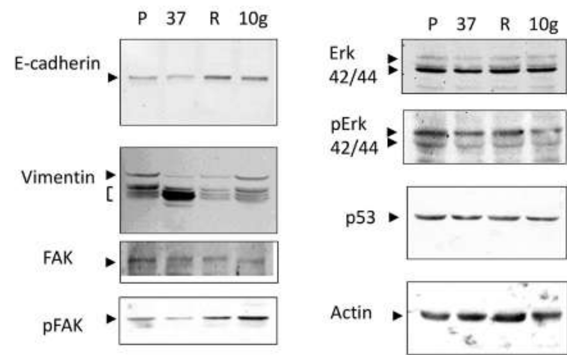


**Fig. 4.** Immunoblotting shows upregulation of E-cadherin in 10 g exposed MCF-7 cells. Immunoblotting of MCF-7 cell extracts was performed to detect expression of E-cadherin, vimentin, FAK, pY576-FAK, Erk, pErk, p53, and actin. The images are annotated to indicate the detected proteins. Images show representative experiments out of 2 performed for each protein. The cells for these tests were recovered as described in the Material and Methods section. Notably, the cells were subjected to a 6-week simulation culture that included 10 days exposure to 10g force for "10g"-cells, followed by a 1-week recovery under standard culturing conditions, and then immunoblotting experiments were performed

## DISCUSSION

Change of the gravity force affects the shape of cells, distribution of intracellular components, and triggers different signaling mechanisms [1, 4]. Here we report that the 10 g exposure of human cancer cells for 10 days promoted the lower rate of formation of 3D structures by cells, lower cell-substrate adhesion, migration, and invasiveness of the cells, as compared to the parental cells (Fig. 1–4). These observations reflect the acquisition of functional properties by the cells that restrict oncogenic features of the parental cells. The lack of cell-cell contact inhibition, enhanced formation of 3D structures, enhanced migration, and invasiveness are associated with actively growing cancer cells. Observed enhanced expression of E-cadherin (Fig. 4) suggests that the cells acquired a ‘stiffer’ phenotype typical for non-aggressive cancerous cells or for cancerous cells undergoing mesenchymal to epithelial transformation [27, 28]. Note that the decrease of E-cadherin expression was observed under exposure to microgravity [5, 6], that is opposite to hypergravity in our experiments that showed increase for E-cadherin. The higher level of FAK phosphorylation (Fig. 4) reflects the increased activity of this kinase. This is in agreement with the involvement of FAK in cell-cell and cell-substrate interactions [9, 27].

MCF-7 human breast adenocarcinoma cells are the well-established model of breast tumorigenesis. These cells were studied in microgravity experiments [5, 6]. Our 10 g hypergravity data and results with microgravity are in concordance with the type of main response of cells by the impact on cell-cell and cell-substrate interactions. To evaluate whether our observations with MCF-7 cells are relevant to other cells, we tested 3 other types of cells (Supplementary Fig. S1-S4). We observed that 10 g exposed ACHN human renal carcinoma and MDA-MB-231 human breast adenocarcinoma cells showed enhanced cell-cell contact inhibition similarly to MCF-7 cells (Fig. 1 and Supplementary Fig. S1). Two types of renal carcinoma cells, ACHN and 786-0, showed similar to MCF-7 adhesion profiles (Fig. 2, *A* and Supplementary Fig. S2). 786-0 showed similar to MCF-7 generation of MTT signal for 10 g exposed cells (Fig. 3, *C* and Supplementary Fig. S4). Combined, these data suggest that presented here MCF-7 results may be observed for other types of cells. Therefore, our supplementary data encourage further study of these cell lines, cells representing different stages of oncogenic transformation and different types of cancer.

A long-term exposure promotes stabilization of the cellular phenotype, while responses to a short-term exposure may revert. A microarray and RNA-Seq study by Vahlensiek et al. [19] demonstrated the full reverse of changes after Jurkat T cells were exposed to 9 g for 15 min only and then cultured under the

normal 1 g culture condition. To exclude transient short-term responses, we opted for a long-term 10-day exposure to 10 g. Moreover, culturing cells at normal 1 g gravity for 1 week after 10 g exposure would revert transient changes. Therefore, in our study MCF-7 cells demonstrated changes that were acquired as relatively stable for at least 6 months after exposure to 10 g.

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**Competing interests**

The authors declare no competing interests in relation to this manuscript.

**Data availability**

All data are freely available upon request. Supplementary Figures S1 to S4 are freely available at FigShare DOI: 10.6084/m9.figshare.17055317.

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## ДОВГОТРИВАЛИЙ ВПЛИВ 10G-ГІПЕРГРАВІТАЦІЇ СТИМУЛЮЄ МІЖКЛІТИННІ КОНТАКТИ ТА ІНГІБУЄ ПРИКРІПЛЕННЯ ДО СУБСТРАТУ, МІГРАЦІЮ ТА ІНВАЗИВНІСТЬ MCF-7 КЛІТИН РАКУ МОЛОЧНОЇ ЗАЛОЗИ ЛЮДИНИ

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***Стан питання*:** Гравітація є фундаментальною силою, яка контролює клітини людини. Рак є однією з 4 основних проблем зі здоров'ям під час довготривалих космічних польотів. У рамках проєкту «Рак в Космосі» досліджують поведінку ракових клітин людини в умовах космічного польоту. Дослідження впливу гравітації є важливою складовою. ***Мета*** цієї роботи полягала в дослідженні впливу 10g-гіпергравітації на онкогенні характеристики клітин аденокарциноми людини MCF-7. ***Матеріали та методи*:** Клітини піддавали навантаженню в 10 g впродовж 10 днів в межах 6 тиж культивування, яке відтворювало умови космічного польоту. Перед подальшими дослідами клітини культивували 1 тиж у стандартних умовах культури клітин. Було виконано тести на проліферацію, виживання, клітинні контакти, інвазивність та міграцію клітин. Експресію білків досліджували методом імуноблотингу. ***Результати*:** Проліферацію, клітинні контакти, формування 3-вимірних структур, міграцію та інвазивність MCF-7 клітин, що зазнали впливу 10 g гіпергравітації, було порівняно з клітинами, культивованими при 1 g. Експоновані за 10 g клітини демонстрували посилення міжклітинних контактів та зниження 3-вимірного росту клітин у щільній культурі. Цей ефект корелював зі зниженням проліферації в щільній культурі при порівнянні з неекспонованими клітинами. Клітини, експоновані при 10 g, також демонстрували зниження міграції, зв'язування із субстратом та інвазивності при порівнянні з неекспонованими MCF-7 клітинами. Було виявлено підвищення експресії Е-кадгерину та фосфорильованої кінази фокальних бляшок (фосфо-pY576-FAK) у клітин, експонованих при 10 g, та відсутність змін у експресії Erk, фосфоErk, FAK та p53. ***Висновок*:** Довготривале експонування клітин MCF-7 в умовах 10 g приводить до посилення міжклітинних контактів та інгібування взаємодії з субстратом, міграції та інвазивності.

***Ключові слова*:** гравітація, гіпергравітація, ракові клітини людини, MCF7, міжклітинна взаємодія, адгезивність клітин.

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