Chapter 9

Tumor Cells in Microgravity

Jun Chen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77214

Abstract

The excessive proliferation and metastasis of tumor cells are due to frequent genetic alterations and subsequent stimulation of abnormal signal transduction pathways. Inventing and improving novel therapeutic strategies are critically needed. However, it remains unknown which of these pathways is essential to tumor initiation and progression. A weightless environment on Earth is a rare phenomenon, achieved using various simulations, but brings about changes of internal cellular structure and interactions among cells not normally seen under normal terrestrial gravitational conditions. For this reason, spaceflight experiments are of great value for cell biology research in general and for cancer research in particular. Many experiments indicate that microgravity, more so actual spaceflight as opposed to simulations, induces changes in the expression and secretion of genes as well as proteins involved in cancer cell proliferation, metastasis, and survival, shifting the cells toward a less aggressive phenotype. Therefore, studies on the biological features and gene expression of tumors cells under microgravity conditions may underline new clues to the tumor initiation, process, diagnosis, and therapy.

Keywords: space, microgravity, morphology, apoptosis, migration, tumor cells

1. Introduction

In the past 40 years, the development of the space industry has made people aware of the effects of microgravity on biological life, including cerebrospinal fluid flow change, body fluid electrolyte loss, muscle atrophy, bone demineralization, and immune system function decline [1]. Similarly, microgravity has been shown to alter some properties of cells, including cell morphology, function, and the cellular response to the environment. Observations from cells in the space environment provide inspiration for our research, in particular, the use of microgravity simulation studies for growth of cancer cells.

The interest in cellular response to microgravity started early in the 1970s, aboard the US Skylab. Research on Skylab included studying the response of humans as well as their cells to microgravity exposure. Similar research continued on future missions and space stations. However, like all astronaut, biological and physiological system research in space, including studying cells and their growth in space, requires precious resources and astronauts' time, which are scarce and in short supply. As a result, ground analogs were developed to test different physiological systems in simulated microgravity, so space agencies developed ways to replicate the microgravity environment in order to culture cells on Earth.

In 1995, Dr. Jessup supplied and successfully cultured cancer cells aboard STS-70 and a few years later aboard STS-80 using the bioreactor to confirm his results. Jessup and his group showed that their flown samples of colon carcinoma cells grew bigger and aggregated better than ground controls, 30 times the volume of the ground controls, and were more representative of cancer seen when growing in the body. These initial studies showed that microgravity was an environment that is favorable to cell growth and differentiation in addition to being more representative of in vivo growth [2].

Earth-based research groups have used the rotating wall vessel (RWV) bioreactor to support the development of several models of cancer cell lines. These include models of breast cancer, cervical cancer, colon cancer, hepatocellular carcinoma, neuroblastoma, melanoma, ovarian cancer, and prostate cancer. The bioreactor was not only a technological innovation, but was also a unique system benefiting biomedical research by allowing cells to be cultured in a 3-D environment on Earth [3]. Other systems have been developed in an attempt to simulate microgravity, such as the random positioning machine (RPM) and magnetic levitation.

Through ground-based simulations and spaceflight research, we know that simulated microgravity has been emerging as a new tool to develop potential therapy for tumor treatment [4]. With the developments from this research, more interest is being paid to the effects of microgravity on tumor cells. Increasing number of investigations has indicated that microgravity has evident effects on the morphology, proliferation, apoptosis, invasion, and migration, along with inhibiting cancerous cell growth and invasion. However, the details of the exact mechanism still elude us and are still being studied.

2. Changes of morphology

It has been found that after microgravity intervention, mesenchymal stem cell morphology changed significantly from spindle to round. "Spirit composing body," shape change will inevitably lead to changes in their function. Under the guidance of the traditional Chinese medicine "Circumference philosophical," the cells with round change are primitive state. The round bone marrow mesenchymal stem cells have greater differentiation potential in microgravity [5] (**Figure 1**). This study uses ground rotary cultivating device (2-D-clinostat) simulation of cells in space (**Figure 2**), using 1G gravity (normal gravity, NG) as a control with simulated microgravity (SMG) intervention of bone marrow mesenchymal stem cells to observe the differentiation into neural cells and endothelial cells. At the same time, cytoskeleton as well as

http://dx.doi.org/10.5772/intechopen.77214

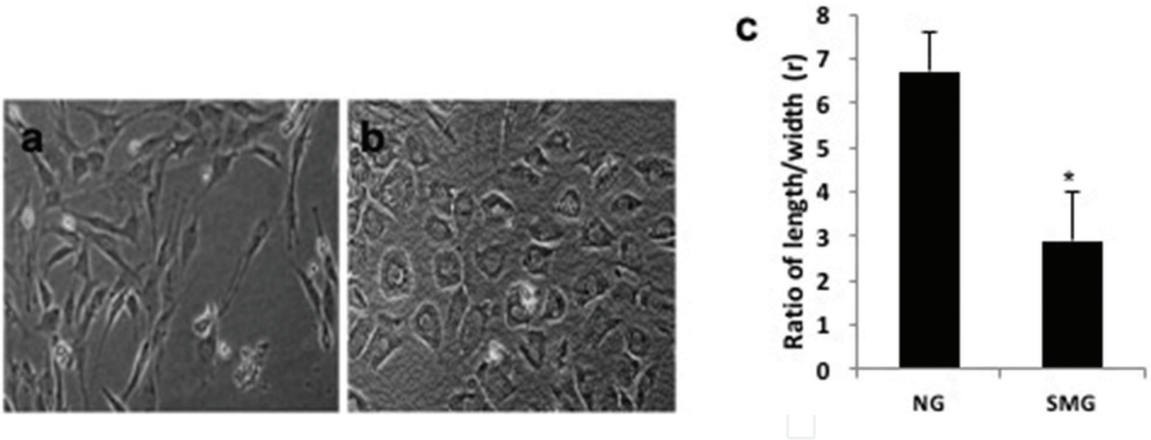

Figure 1. Phase-contrast microscopic analysis of the effect of SMG (b) on the morphology of BMSCs compared to NG (a). (c) The changes of the ratio of the width/length of BMSCs in the different groups. * denotes $P < 0.05$, versus the NG group.

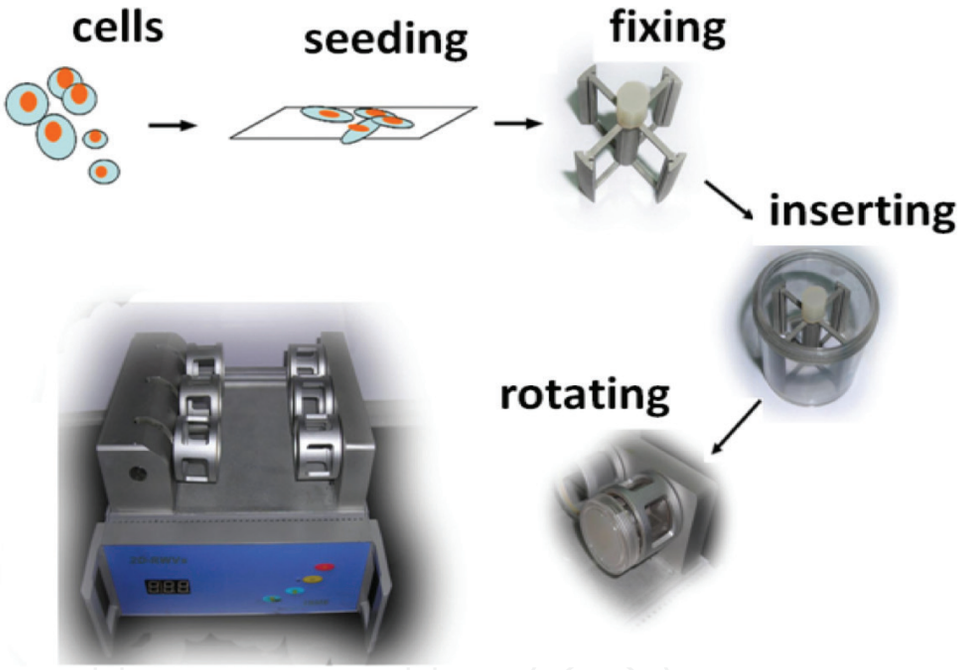

Figure 2. A clinostat or rotating vessel was used to form the weightless environment to prevent the cell from feeling gravity; the machines are based on the theory that sensing no gravity would have similar effects as being microgravity. The clinostat model system (clinorotation) used in this study tends to provide an average of zero vector in the apparent gravity on the cell culture.

the key molecule of RhoA activity is observed, thereby directing the possible differentiation mechanism of stem cells.

Other than stem cells, real and simulated microgravity induces early alterations of the cytoskeleton in different kinds of human cells, such as thyroid cancer cells [6, 7], endothelial cells [8], and glial cells [9].

Our previous works have concluded that modeled microgravity causes changes in the cytoskeleton and focal adhesions in malignant human MCF-7 cells [10].

Microtubules, which are components of the cytoskeleton that are involved in maintaining the structure of the cell, were disrupted in MCF-7 cells within 4 h of SMG. Instead of long, strongly labeled microtubules in NG groups, only a few fibers could be distinguished against the strong background (**Figure 3**). Self-organization of microtubules into stationary macroscopic patterns is gravity-dependent, and the patterns correspond to different microtubule orientations [11]. Also, the formation of radial microtubule arrays depends mostly on the activity of centrosome. Lewis et al. reported that microtubule organizing centers (MTOCs) were poorly defined in SMG [12]. It is obvious that the anchoring between microtubule and the centrosome is very complicated; so, many studies targeted the function of cytoplasmic dynein and its cofactor dynactin. Dynein transfers some centrosomal proteins to the centrosome and reorganizes radial microtubule arrays for cell division. Thus, we hypothesize that the disorganization of microtubule fibers and their reestablishment might be associated with MTOCs modification.

The cytoskeleton is made up of actin microfilaments, tubulin microtubules, and vimentin intermediate filaments [13]. The F-actin cytoskeleton has been involved in changes in cell morphosis and function as well as signaling path under weightless conditions. It was revealed that the amount of F-actin in A431 epidermoid carcinoma cells increased under real microgravity for

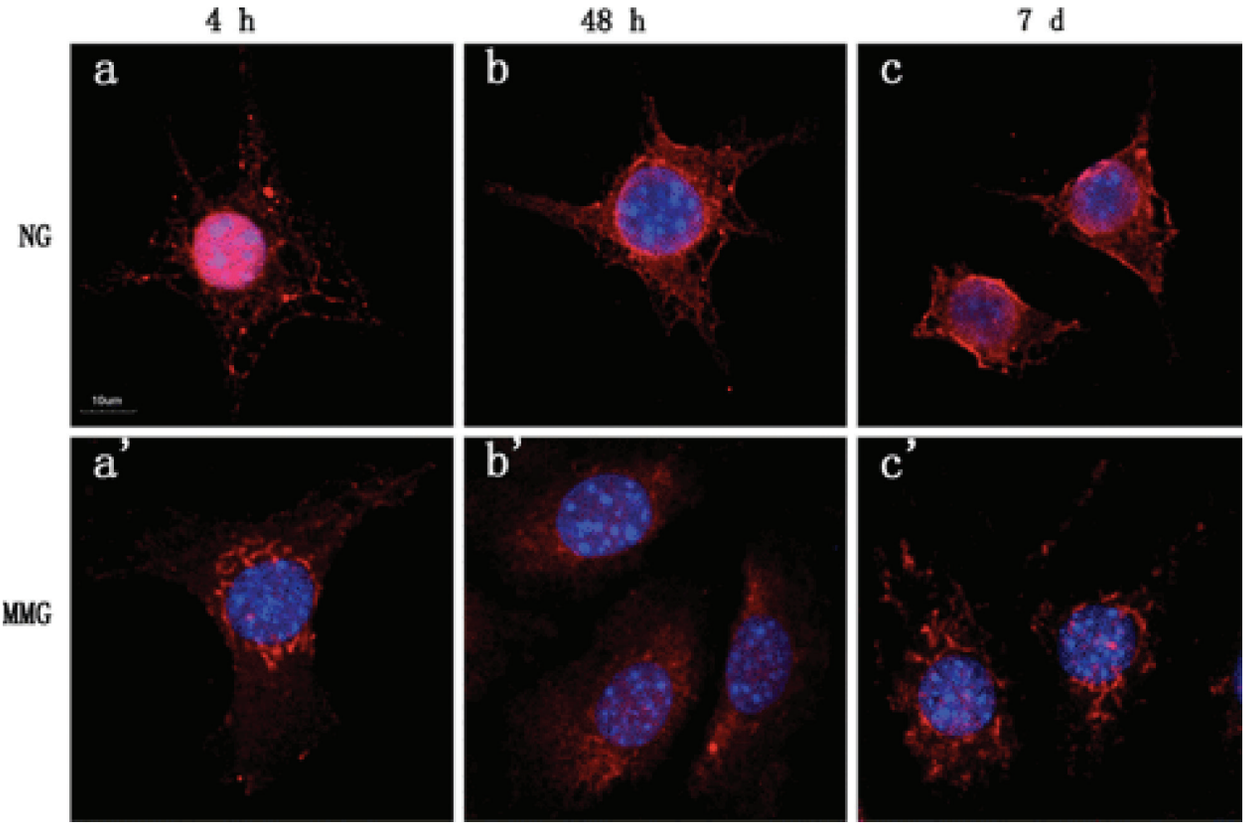

Figure 3. Microtubules formation of MCF-7 cells is changed in MMG. Scale bar is 10 μm. Visualization of β-tubulin (TRITC; red) and nuclear chromatin (Hochest; blue). It is shown that microtubules keep radiation from the perinuclear area toward the cell periphery in control cells as time varying. (a–c) (4 h, 48 h, and 7 days, respectively). While in the MMG groups, altered fibers and short microtubules filaments could be observed. (a′–c′) (4 h, 48 h, and 7 days, respectively).

7 min [14]. This leads us to conclude that the actin microfilament structure is sensitive to gravity and that rebuilding of cytoskeleton may affect signal transduction [14]. Thyroid cancer cells were flown in a parabolic flight mission and they detected early alterations in the actin microfilaments. After 22-s microgravity stimulation, F-actin changed significantly, and the human beta actin (ACTB) expression was strikingly upregulated after the 1st and 31st parabolas [15].

Actin monomers polymerize only onto the existing barbed ends of the actin array, so that the actin filaments can be elongated to the cell periphery. In contrast to NG samples, microfilaments did not form preferential orientation with less labeled lamellipodia. Cell microfilament bundles are assembled primarily by bundling of preexisting actin filaments [16]. It has been reported that tension development in the preexisting actin cytoskeleton is critical for the formation of stress fibers [17]. Tension would act on mechanical connections of the actin filaments to reorganize a kind of meshwork. However, spaceflight or stimulated weightlessness would destroy the intracellular prestress and tension balance, and eventually cells show irregular formation of cytoskeletal actin filaments [18].

3. Changes of cell growth, cycle, and apoptosis

Glioma is the most common and aggressive form of cancer of the central nervous system with a median survival time of 15 months and a 5-year survival about 5% after initial diagnosis [19]. Despite the standard of therapy available, including maximal safe surgical resection, radiotherapy, and temozolomide (a form of chemotherapy) [20], nearly all patients relapse. The excessive proliferation and metastasis of glioma is due to frequent genetic alterations and subsequent stimulation of abnormal signal transduction pathways [21, 22]. Inventing and improving novel therapeutic strategies are critically needed. However, it remains unknown which of these pathways is essential to glioma initiation and progression.

To assess the effect of SMG on glioma growth, U251 cells were cultured under either ground condition or SMG (SM-31 random locator applied) for different time periods (0, 12, 16, 20, 24, 36, 48, 72, and 96 h). CCK8 measurements, a chemical kit employed to measure cell proliferation, showed that SMG inhibited U251 cells activity in a time-dependent manner. The more time handled, the less activity the U251 cells had. For 48~96 h, SMG markedly induced cell death of U251, cell activity decreased maximum about 45%. Therefore, we chose 72-h timepoint to use in the following experiments. This was further confirmed by our data derived from fluorescence-activated cell sorter (FACS) analysis via annexin V-FITC and PI double staining to investigate apoptosis of U251 cells exposed to SMG for 72 h, which showed 2.4% of control cells stained positive for both annexin V and PI, representing a minor subpopulation undergoing a spontaneous apoptosis. After being cultured in SMG for 72 h, apoptotic subpopulation increased to 29.3%, indicating that SMG promoted cell death via apoptosis. Western blot (WB) test confirmed that apoptotic promoting proteins cleaved-caspase 3 and cleaved-caspase 9 were markedly upregulated at 48 h by SMG compared with NG condition. Meanwhile, U251 cells' metabolic activity was detected by β-Gal Staining Set, blue-stained cells significantly increased by SMG, about 30% increase compared with NG condition. FACS analysis further demonstrated that SMG treatment led to 14.11 ± 1.73% of U251 cells arrested at G2/M phase (**Figure 4**).

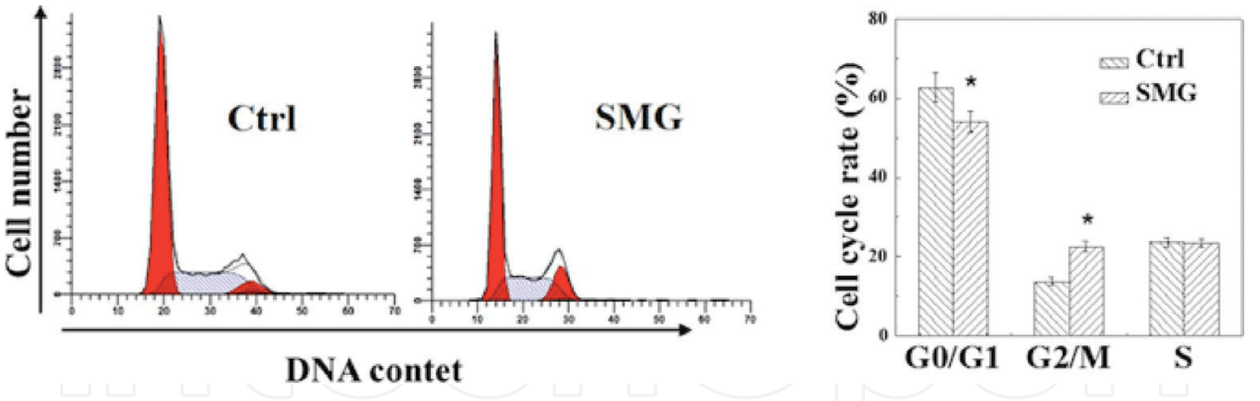

Figure 4. Cell cycle was analyzed by flow cytometry after PI staining. The bar graph represents the number of the cells in different phases.

Caspases are a family of cysteine proteases. Most caspases play a central role in activating cell apoptosis by cleaving selected target substrates in a cysteine-dependent aspartate-directed manner. To further dissect molecular mechanisms of SMG-induced glioma apoptosis, WB analysis was performed to confirm the level of apoptotic protein, Bcl2, Bnip3, pro-caspase 3, cleaved-caspase 3, pro-caspase 9, and cleaved-caspase 9. Zhao et al. found that SMG promotes BL6-10 melanoma cell apoptosis through downregulating Bcl2 and Bnip3 and upregulating caspases 3/7/8 [23]. We showed that two antiapoptotic proteins Bcl2 and Bnip3 were downregulated, while cleaved-caspase 3 and cleaved-caspase 9 were upregulated in cells under microgravity conditions, respectively, leading to enhance glioma cell death.

Never in mitosis gene A (NIMA)-related kinase 2 (Nek2) is one of multiple cell cycle-regulated protein kinases that localizes to the centrosome and is required for mitotic progression and correct bipolar spindle formation [24]. Upregulated Nek2, which is confirmed in a number of neoplastic diseases, including prostate cancer, lung cancers, colorectal cancer [25], and myeloma, also exhibits adverse correlation with overall survival of multiple malignancies [26]. All of this suggests that Nek2 may regulate the metastasis of glioma, although few reports have shown this. In our unpublished study, Nek2 may play a crucial function in SMG-treated U251cells, which arrested at the mitotic phase (chromosome separation).

Nek2 is implicated in centrosome separation and is reported to displace linker proteins from centrosomes through phosphorylation at the beginning of mitosis, and Nek2 phosphorylates the centrosomal linker proteins C-Nap1 and rootletin resulting in linker dissociation. hSav1-Mst2-Nek2 centrosome disjunction pathway becomes essential for bipolar spindle formation, Nek2 kinase to regulate centrosome disjunction. We found SMG suppressed expression of Nek2 and distances between two centrioles by γ-tubulin staining. The experimental results were consistent as Di Agostino et al. [27].

4. Changes of migration

The formation of a protrusion initiates the cell migration cycle process, but the protrusions need to be stabilized to the substratum so as to move forward. The process of continuous

coordinated formation and disassembly of adhesions is crucial for migration. These adhesion sites, named as FAs, serve as traction points to propel the cell forward. Therefore, the important mechanism for creating cell movement includes the maturation of FAs. FAs become mature during the binding and clustering of integrins and function physically as a link of the cytoskeleton to the extracellular matrix (ECM) [28]. This correlation between cytoskeleton and FAs in structure underlines the possibility that FAs formation might be changed during microgravity. In order to explore the possible mechanism, we examined FAs formation by vinculin immunofluorescence. Vinculin is one of the most prevalent elements in FAs. It seems that vinculin facilitates the assembly of FAs by interacting and recruiting its various partners [29]. We demonstrated that microgravity disorganized FAs of MCF-7 cells via quantifying FAs parameters. After 7 days stimulation of SMG, the amount of FAs was still low by detecting topographical adhesion parameters and showed no change as time varied. We therefore could conclude that FAs created in microgravity were less mature than those established in normal gravity. Fewer and smaller FAs can lead to the weaker cell spreading and migrating. Thus, we speculated that abnormal FAs structure in MCF-7 cells under SMG may contribute to the change of cell migration [10].

Since FAs also contain a lot of growth factor receptors, kinases, and signaling proteins, FAs have been referred as localized sites converging growth factor and adhesion signaling. The integrin family is the major transmembrane ECM receptors in these sites. FAK, PYK2, and ILK are well known as the key effectors in FAs signaling and a potential integrator of inhibition of MCF-7 cell migration by modeled microgravity. Because the changes of morphological and topographical cytoskeletal structures of FAs were observed in MCF-7 cells, we speculated that activities of the kinases would be altered under clinorotation conditions. Here, our hypothesis was confirmed by observing a decrease in FAs kinases phosphorylation level (FAK, PYK2, and ILK) in contrast to NG controls, while there was no significant change in total FAK, ILK, and PYK2 protein expression in both the NG controls and SMG groups. The effects of microgravity on the suppression of FAs kinases activity in our experiments were in accordance with other reports, though they were observed in different cells by different devices, which could suggest that SMG might suppress the FAs kinases activity in various kinds of cells [30].

As we know, integrins play a critical role in cell adhesion and migration. We explored that SMG could decrease the expression of integrins, a downregulation of integrins (integrinβ1 and integrinβ4) at both protein and mRNA levels after SMG compared to controls. Downregulated integrins in SMG are not a new finding, and it has been reported that SMG suppressed integrins (αv, α5,β1) expression in MG-63 cell. Via binding to the ECM, phosphorylation of signaling proteins at FAs was triggered, such as FAK, ILK, and PYK, whereby protein kinase C (PKC) and GTPases pathways were activated. The small G proteins, Rho family regulate the rebuilding of actin fibers and FAs formation, by which determines cell movement. In fact, the previous studies have reported that rho activation in the endothelial cells and MSCs could be affected by simulated microgravity. Also, it has been found repeatedly that microgravity modulates PKC signaling in neurons. Activation of PKC could directly induce cells' motility and migration. Furthermore, it is generally accepted that the releasing from the RhoGDI-1 molecule is required for activation of RhoGTPases. PKCα is confirmed to phosphorylate RhoGDI-1 and then catalyze the release of bound GTPases [31]. No doubt, the

pathways regulating cell migration are very complicated and seem to vary in different cells and different species. In general, it seems that decreased integrin expression and downregulated FAs kinase activity are an essential step in suppression of cell migration by SMG.

5. Changes of genes

To investigate the involved mechanisms in more detail, gene level researches are expected to yield novel targets for cancer therapy, which may then be exploited in the form of new chemotherapeutics.

Dr. Xiao Ma cultured thyroid cancer cells in space (Shenzhou 8 space mission) on a random positioning machine (RPM) for 10 days to evaluate differences between real and SMG. About 2881 genes were regulated during 10 days of cell exposure to microgravity [32]. These genes were subdivided into different clusters (**Figure 5**) that allowed us to distinguish the difference between SMG (RPM) and spaceflight effects. However, two clusters of genes expressed similarly under either real or SMG. This research demonstrated that the effects of RPM and spaceflight both exert 3-D growth, but may not change gene expression in the same direction.

For the sake of the very limited amount of cells returned from space, the combination of gene expression and secretion analysis was detected on the cytokine, protease, and kinases factors that may play a pivotal role in the development of metastases. The list was made up of IL6, IL8, IL15 (interleukin family), OPN (osteopontin), VEGFA, VEGFD (vascular endothelial growth factor), and FGF17 (fibroblast growth factor).

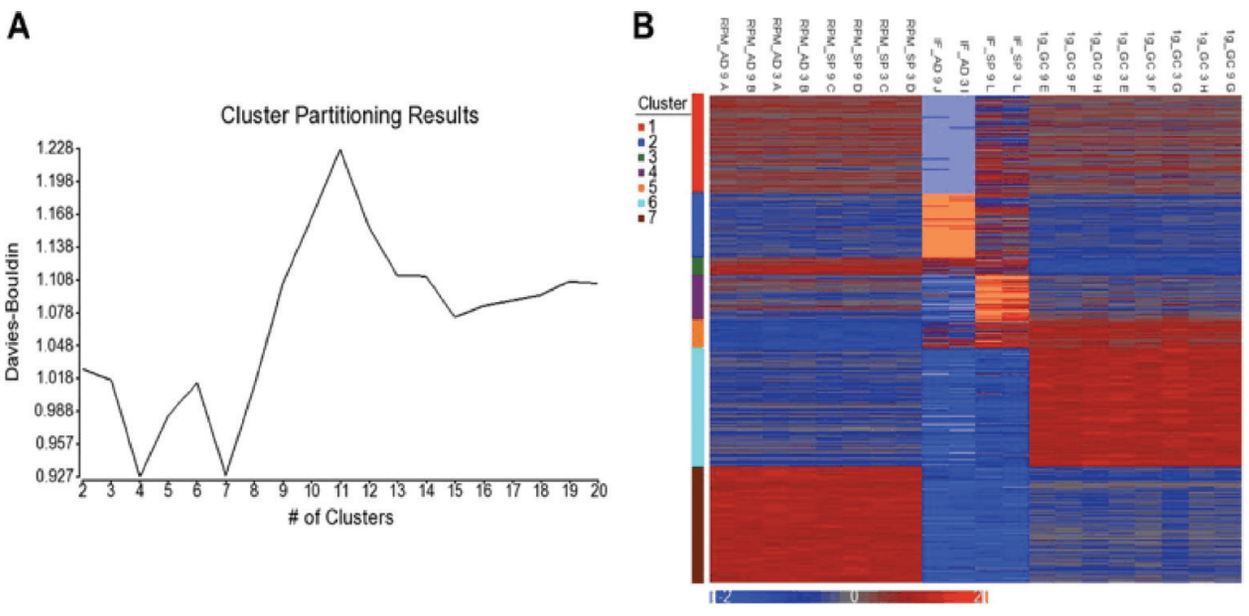

Figure 5. (A) Gene array analyses on FTC-133 cells cultured for 10 days under both conditions. ANOVA resulted in 2881 twofold-regulated probes. The Davies-Bouldin cluster estimation resulted in a local minimum of seven clusters. (B) RPM-specific effects (clusters 5 and 7), spaceflight-specific AD cell and MCS effects (clusters 1, 2, and 4), and general gravity effects (clusters 3 and 6) were shown in gene map. An outstanding expression level of spaceflight samples was found in contrast to the moderate levels in RPM samples and controls.

In this research, VEGFA and VEGFD, which are members of the vascular endothelial growth factor (VEGF) family, were also observed. It is well known that VEGFA is the most important growth factor driving angiogenesis and is also implicated in many processes like tumor vascularization, wound healing, and burn injury. VEGFA expression has been found to increase in papillary thyroid carcinoma in contrast to that in healthy patients [33]. While on the other hand, VEGFD serum levels were found to decrease in patients with thyroid cancer [34]. In this study, an obvious decrease in expression of VEGFA was confirmed in the RPM as well as the spaceflight samples, while no difference was observed between adherent (AD) cells or multicellular spheroids (MCSs). Similarly, VEGFA secretion was also reduced on the RPM, but no influence in real microgravity. Furthermore, VEGFD gene expression upregulated in cells cultivated on the RPM as well as in space. All these results seem to get a conclusion that microgravity exerts a shift of the thyroid tumor cells to a more benign, less metastatic phenotype.

Interestingly, the similar tendency was observed with the interleukins in microgravity. IL6 and IL8, encoded by the IL6 and IL8 genes, are involved in tumor cell growth, metastasis, and angiogenesis. IL15, on the contrary, is able to activate several antitumor mechanisms, such as activating CD8 T cells to kill tumor cells [35]. Furthermore, it has been found that IL15 was effective in several tumor therapy experiments [36]. Their results explored a significant downregulation of IL8 gene expression in both simulated and real microgravity cells, which is also in accordance with the IL8 secretion pattern. The weightless effect of reducing IL6 gene expression was only observed in adherent cells, the same to that described for endothelial cells [37].

Of note, both IL6 and IL8 gene expression were strikingly upregulated during a parabolic flight. The study also showed earlier that IL6 and IL8 were most possibly implicated in the gravity-sensitive signaling required for spheroid formation. It seems that this signal pathway is attenuated after exposure to microgravity and that the tumor cells are shifted toward a less aggressive biological behavior. This hypothesis was also confirmed by the observations for IL15. IL15 gene expression increased only slightly on the RPM and during parabolic flight, but they explored a strong increase in the MCSs during spaceflight. Thus, we speculate that microgravity triggers some antitumor pathways involving IL15 and, as MCSs resemble tumors in their 3-D structure, the cell-cell interactions should be affected.

6. Conclusion

Taken together, it is no doubt that spaceflight experiments are of great value for cell biology research, especially for cancer research. The previous studies indicate that microgravity, both actual and simulated microgravity, induces changes in cancer cell proliferation, metastasis, and survival, bringing the cells toward a less aggressive phenotype. This effect is greater in actual spaceflight; however, ground-based simulation has been shown to be an essential tool in our understanding of gravity and it effects on a cellular level in cancer cells. The above body shows that not only does the architecture of tumor cells change in microgravity, but the cell function and gene expression also are different. The changes of some gene expression reorganize the cytoskeleton which influences the cell growth, migration, and apoptosis. And some other genes impress tumor cell by regulating the immune antitumor pathway.

However, most of the studies are in vitro, and the evidence from human or animals is much rare. Is the microgravity strategy safe for human? How to create microgravity environment in body? Those are the thorny tissue for clinical translation. With the recent discoveries of nanomagnetic fluids, it suggests an innovative method of treating tumors using magnetic fluid-modeled microgravity. Magnetic fluids are delivered by outside magnetic field to tumor tissue either intravenously or through direct injection, and this is followed by application of a uniform external magnetic field that causes microgravity. The concept of magnetic fluid-modeled microgravity to treat tumor is novel, and the technology involved is simple, economical, and might be suitable for clinical applications in future.

All in all, as new information about the biology of cancer emerges, treatments will be developed and modified to increase effectiveness, precision, survivability, and quality of life. It is still a long shot to take advantage of microgravity as a suitable way to treat tumor.

Acknowledgements

The present chapter was funded by the National Natural Science Foundation of China (grant no. 81402055), and Youth Science and Technology Innovation Personnel Training Project of Shaanxi Province (grant no.2017KJXX-84).

Author details

Jun Chen

Address all correspondence to: chj2002819@163.com

Department of Encephalopathy, Traditional Chinese Medicine Hospital of Shaanxi Province, Xi'an, Shaanxi, China

References

[1] Sonnenfeld G. Space flight, microgravity, stress and immune responses. Advances in Space Research. 1999;**23**:1945-1953

[2] Jessup JM, Goodwin TJ, Garcia R, Pellis N. STS-70: First flight of EDU-1. In Vitro Cellular & Developmental Biology. 1996;**32**:13A

[3] Hjelm BE, Berta AN, Nickerson CA, Arntzen CJ, Herbst-Kralovetz MM. Development and characterization of a three-dimensional organotypic human vaginal epithelial cell model. Biology of Reproduction. 2009;**82**:617-627

[4] Jun C, Zhiqiang Y, Rongrong L, Nanding W, Jing L, Zongren W. Magnetic fluid-modeled microgravity: A novel way to treat tumor. Medical Hypotheses. 2011;**77**:953-955. DOI: 10.1016/j.mehy.2011.07.053

[5] Wang ND, Wang H, Chen J, Zhang XF, Xie J, Ma J, Wang W, Wang ZR. The simulated microgravity enhances multipotential differentiation capacity of bone marrow mesenchymal stem cells. Cytotechnology. 2014;**66**:119-131. DOI: 10.1007/s10616-013-9544-8

[6] Grimm D, Kossmehl P, Shakibaei M, Schulze-Tanzil G, Pickenhahn H. Effects of simulated microgravity on thyroid carcinoma cells. Journal of Gravitational Physiology. 2002;**9**:253-256. DOI: 10.1096/fj.01-0673fje

[7] Grimm D, Bauer J, Kossmehl P, Shakibaei M, Scho ̈nberger J. Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. The FASEB Journal. 2002;**16**:604-606. DOI: 10.1096/fj.01-0673fje

[8] Grimm D, Bauer J, Ulbrich C, Westphal K, Wehland M. Different responsiveness of endothelial cells to vascular endothelial growth factor and basic fibroblast growth factor added to culture media under gravity and simulated microgravity. Tissue Engineering. Part A. 2010;**16**:1559-1573. DOI: 10.1089/ten

[9] Uva BM, Masini MA, Sturla M, Prato P, Passalacqua M. Clinorotation-induced weightlessness influences the cytoskeleton of glial cells in culture. Brain Research. 2002;**934**:132-139. DOI: 10.1016/s0006-8993(02)02415-0

[10] Li J, Zhang S, Chen J, Du TY, Wang YC, Wang ZR. Modeled microgravity causes changes in the cytoskeleton and focal adhesions, and decreases in migration in malignant human MCF-7 cells. Protoplasma. 2009;**238**:23-33. DOI: 10.1007/s00709-009-0068-1

[11] Papaseit C, Pochon N, Tabony J. Microtubule self-organization is gravity-dependent. Proceedings of the National Academy of Sciences. 2000;**97**:8364-8368. DOI: 10.1073/pnas.140029597

[12] Lewis ML, Reynolds JL, Cubano LA, Hatton JP, Lawless BD, Piepmeier EH. Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). The FASEB Journal. 1998;**12**:1007-1018

[13] Benjamin M, Archer CW, Ralphs JR. Cytoskeleton of cartilage cells. Microscopy Research and Technique. 1994;**28**:372-377

[14] Boonstra J. Growth factor-induced signal transduction in adherent mammalian cells is sensitive to gravity. The FASEB Journal. 1999;**13**:35-42

[15] Ulbrich C, Pietsch J, Grosse J, Wehland M, Schulz H. Differential gene regulation under altered gravity conditions in follicular thyroid cancer cells: Relationship between the extracellular matrix and the cytoskeleton. Cellular Physiology and Biochemistry. 2011;**28**:185-198. DOI: 10.1159/000331730

[16] Hirata H, Tatsumi H, Sokabe M. Dynamics of actin filaments during tension-dependent formation of actin bundles. Biochimica et Biophysica Acta. 2007;**1770**:1115-1127. DOI: 10.1016/j.bbagen.2007.03.010

[17] Kolega J. Effects of mechanical tension on protrusive activity and microfilament and intermediate filament organization in an epidermal epithelium moving in culture. The Journal of Cell Biology. 1986;**102**:1400-1411

[18] Ingber DE. Tensegrity-based mechanosensing from macro to micro. Progress in Biophysics and Molecular Biology. 2008;**97**:163-179. DOI: 10.1016/j.pbiomolbio.2008.02.005

[19] Ostrom QT, Gittleman H, Fulop J. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. Neuro-Oncology. 2015;**17**:1-62. DOI: 10.1093/neuonc/nov189

[20] Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. The New England Journal of Medicine. 2005;**352**:987-996

[21] Dong Z, Lei Q, Yang R, Zhu S, Ke XX, Yang L, Cui H, Yi L. Inhibition of neurotensin receptor 1 induces intrinsic apoptosis via let-7a-3p/Bcl-w axis in glioblastoma. British Journal of Cancer. 2017;**116**:1572-1584. DOI: 10.1038/bjc.2017.126

[22] Sturm D, Bender S, Jones DT, Lichter P, Grill J, Becher O, Hawkins C, Majewski J, Jones C, Costello JF, Iavarone A, Aldape K, Brennan CW, Jabado N, Pfister SM. Paediatric and adult glioblastoma: Multiform (epi)genomic culprits emerge. Nature Reviews. Cancer. 2014;**14**:92-107. DOI: 10.1038/nrc3655

[23] Zhao T, Tang X, Umeshappa CS, Ma H, Gao H, Deng Y, Freywald A, Xiang J. Simulated microgravity promotes cell apoptosis through suppressing uev1a/ticam/traf/nf-κb-regulated anti-apoptosis and p53/PCNA- and ATM/ATR-Chk1/2-controlled DNA-damage response pathways. Journal of Cellular Biochemistry. 2016;**117**:2138-2148. DOI: 10.1002/jcb.25520

[24] Bayliss R, Fry A, Haq T, Yeoh S. On the molecular mechanisms of mitotic kinase activation. Open Biology. 2012;**2**:120136. DOI: 10.1098/rsob.120136

[25] Xia J, Franqui Machin R, Gu Z, Zhan F. Role of Nek2A in human cancer and its therapeutic potentials. BioMed Research International. 2015;**2015**:862461. DOI: 10.1155/2015/862461

[26] Fang Y, Zhang X. Targeting Nek2 as a promising therapeutic approach for cancer treatment. Cell Cycle. 2016;**15**:895-907. DOI: 10.1080/15384101.2016.1152430

[27] Di Agostino S, Fedele M, Chieffi P, Fusco A, Rossi P, Geremia R, Sette C. Phosphorylation of high-mobility group protein A2 by Nek2 kinase during the first meiotic division in mouse spermatocytes. Molecular Biology of the Cell. 2004;**15**:1224-1232. DOI: 10.1091/mbc.E03-09-0638

[28] Zamir E, Geiger B. Molecular complexity and dynamics of cell-matrix adhesions. Journal of Cell Science. 2001;**114**:3583-3590

[29] Cox EA, Huttenlocher A. Regulation of integrin-mediated adhesion during cell migration. Microscopy Research and Technique. 1998;**43**:412-419

[30] Meyers VE, Zayzafoon M, Gonda SR, Gathings WE, McDonald JM. Modeled microgravity disrupts collagen I/integrin signaling during osteoblastic differentiation of human mesenchymal stem cells. Journal of Cellular Biochemistry. 2004;**93**:697-707. DOI: 10.1002/jcb.20229

[31] Mehta D, Rahman A, Malik AB. Protein kinase C-alpha signals rho-guanine nucleotide dissociation inhibitor phosphorylation and rho activation and regulates the endothelial cell barrier function. The Journal of Biological Chemistry. 2001;**276**:22614-22620. DOI: 10.1074/jbc.M101927200

[32] Ma X, Pietsch J, Wehland M, Schulz H, Saar K, Hübner N, Bauer J, Braun M, Schwarzwälder A, Segerer J, Birlem M, Horn A, Hemmersbach R, Waßer K, Grosse J, Infanger M, Grimm D. Differential gene expression profile and altered cytokine secretion of thyroid cancer cells in space. The FASEB Journal. 2014;**28**:813-835. DOI: 10.1096/fj.13-243287

[33] Hsueh C, Lin JD, Wu IC, Chao TC, Yu JS, Liou MJ, Yeh CJ. Vascular endothelial growth factors and angiopoietins in presentations and prognosis of papillary thyroid carcinoma. Journal of Surgical Oncology. 2011;**103**:395-359. DOI: 10.1002/jso.21844

[34] Nersita R, Matrone A, Klain M, Scavuzzo F, Vitolo G, Abbondanza C, Carlino MV, Giacco V, Amato G, Carella C. Decreased serum vascular endothelial growth factor-D levels in metastatic patients with differentiated thyroid carcinoma. Clinical Endocrinology. 2012;**76**:142-146. DOI: 10.1111/j.1365-2265

[35] Barry M, Bleackley RC. Cytotoxic T lymphocytes: All roads lead to death. Nature Re views. Immunology. 2002;**2**:401-409. DOI: 10.1038/nri819

[36] Zhang M, Yao Z, Dubois S, Ju W, Müller JR, Waldmann TA. Interleukin-15 combined with an anti-CD40 antibody provides enhanced therapeutic efficacy for murine models of colon cancer. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:7513-7518. DOI: 10.1073/pnas.0902637106

[37] Grenon SM, Jeanne M, Aguado-Zuniga J, Conte MS, Hughes-Fulford M. Effects of gravitational mechanical unloading in endothelial cells: Association between caveolins, inflammation and adhesion molecules. Scientific Reports. 2013;**3**:1494. DOI: 10.1038/srep01494

IntechOpen