Chapter 5
Cell Biology in Space

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Abstract This chapter provides an overview of experiments conducted in space and on Earth using machines created to simulate microgravity. Today, research in space on the International Space Station (ISS) or in orbit, as well as the exploration by humans of extraterrestrial environments like the Moon or Mars, is of worldwide interest. The commercial use of space and future space tourism will further increase this interest. The space travels of European astronauts have contributed to this great success with their enormously positive PR activities before, during and after their respective missions.

In the past, space medicine and gravitational biology were disciplines familiar only to a small research community, but they are attracting a lot of interest today. A large number of exciting research findings have been discovered in the last 40 years. Today we know that microgravity has an enormous influence on the biology of human cells, in particular on cellular morphology, the cytoskeleton and growth behavior. Moreover, it changes various biological processes in human cells.

Keywords Microgravity • Human Cells • Cytoskeleton • Multicellular Spheroids • Extracellular Matrix

5.1 Introduction

Analysis of the cellular response to real microgravity in space offers new aspects in cell biology, tissue engineering and cancer research. Studies of the cellular response to microgravity have revealed novel adaptive mechanisms. Growing cells in a microgravity environment induces a three-dimensional (3D) growth behavior in different cell types more closely representing the *in vivo* situation in the human body (Grimm et al. 2014). This chapter summarizes data regarding space experiments conducted aboard the space shuttle, *MIR*, and the International Space Station (ISS). In addition, experiments obtained aboard unmanned space missions (SIMBOX/Shenzhou-8), rocket missions or parabolic flight missions will be discussed (Fig. 5.1). To compare and validate these findings, experiments using so-called ESA ground-based facilities,

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Fig. 5.1 Platforms to provide real microgravity. (**a**) Shenzhou-8 rocket; (**b**) Parabolic Flight Plane Airbus A300; (**c**) TEXUS rocket launch at ESRANGE, Kiruna, Swedens

such as the Random Positioning Machine (RPM), the 2D clinostat and the NASA-developed Rotating Wall Vessel (RWV) bioreactor, will be evaluated (Fig. 5.2).

Long-term space missions induce a variety of health problems in astronauts, cosmonauts and taikonauts (White and Averner 2001; Grimm et al. 2011, 2016). Examples of these are bone loss, osteoporosis and cardiac atrophy together with hypotension and arrhythmias, muscle atrophy or a dysfunction of the immune system (White and Averner 2001; Grimm et al. 2011, 2016). In addition, visual problems are considered to be a major complication of spaceflights. One relevant health concern is the dysfunction of the immune system. This can result in opportunistic infections, or a poor wound-healing process.

One of the main aims of the current research on space medicine is to evaluate the effects of microgravity on human cells. Therefore, investigations of the primary molecular mechanisms of how microgravity might affect cell signaling are currently of interest.

5.2 Human Adult Retinal Pigment Epithelium Cells

A long-term stay in orbit can affect the eyes and might result in visual impairment for space travellers. Identification of the underlying mechanisms is very important. Studies of astronauts revealed spaceflight-induced ocular changes such as choroidal folds, optic disk edema, globe flattening and hyperopic shifts (Mader et al. 2011). It has been hypothesized that these visual problems are connected to cephalad fluid

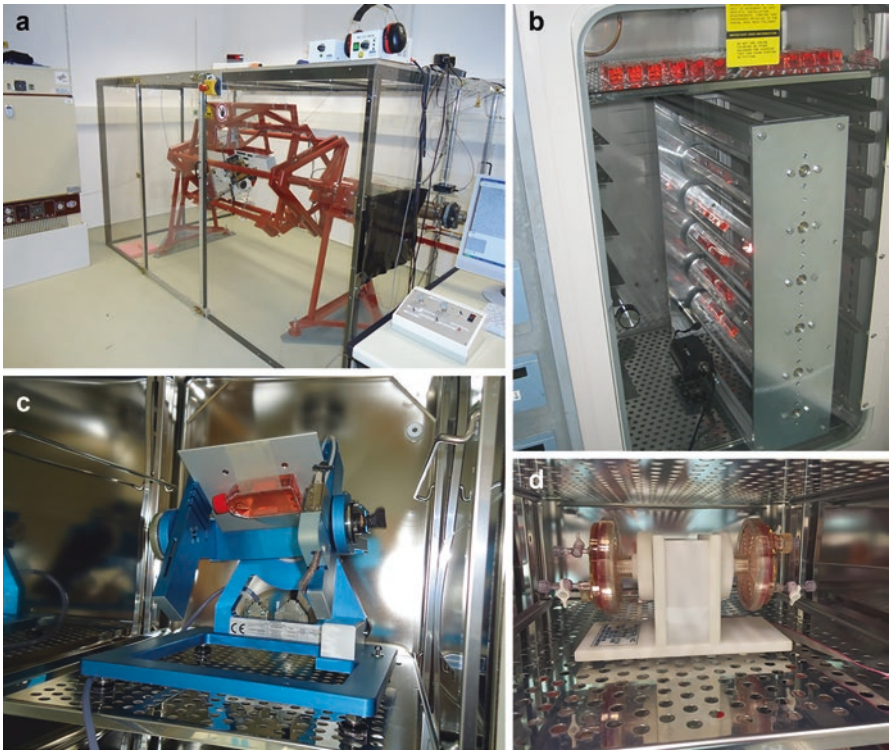

Fig. 5.2 ESA ground-based facilities: (**a**) 2D clinostat microscope for the observation of samples during fast rotation around one axis perpendicular to gravity (Group PD Dr. Ruth Hemmersbach, Gravitational Biology, DLR Cologne, Germany); (**b**) Fast rotating 2D clinostat in an incubator with samples (constructed by PD Dr. Ruth Hemmersbach's group, Gravitational Biology, DLR Cologne); (**c**) Desktop Random Positioning Machine (Airbus, Defense & Space; former Fokker Space, Leiden, NL); and (**d**) Rotating Wall Vessel in an incubator with a chondrocyte experiment (Synthecon Inc., Houston, TX, USA, delivered by Cellon SA, Bascharage, 4940 Schouweiler, Luxembourg)

shifts, intracranial pressure and optic nerve sheath compartment syndrome, as a consequence of longterm microgravity exposure (Zwart et al. 2016; Mader et al. 2016).

The risk of visual impairment is an important health concern for NASA. We had recently examined the effects of simulated microgravity on human adult retinal epithelium cells (ARPE-19 cells). This study showed alterations in the cytoskeleton of ARPE-19 cells (Fig. 5.3). This was paralleled by changes in cell growth and the expression patterns of selected genes involved in cell structure, shape, adhesion, extracellular matrix, migration and angiogenesis (Corydon et al. 2016a).

5.3 Lymphocytes Cultured Under Conditions of Microgravity

A dysfunction of the immune response of astronauts is already evident after a few days in space and after longterm space flights (Pietsch et al. 2011).

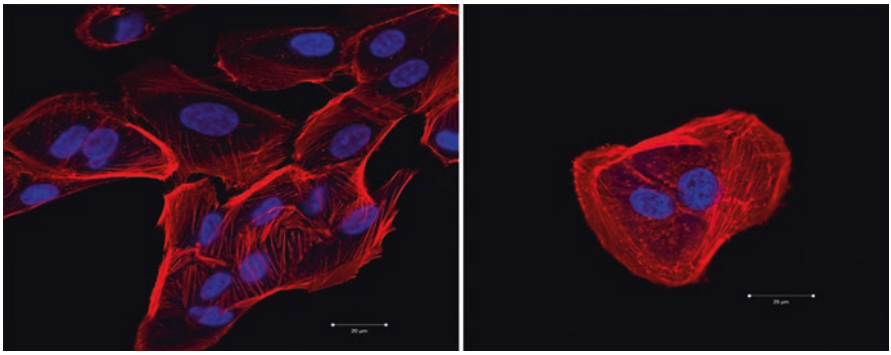

Fig. 5.3 Confocal laser scanning microscopy of rhodamine-phalloidin-stained ARPE-19 cells grown under conditions of 1*g* and after RPM-exposure. Blue staining: DAPI (4′,6-diamidino-2-phenylindole) highlights the nucleus; red staining: rhodamine-phalloidin to visualize the F-actin

Therefore, many researchers have demonstrated in the course of nearly 40 years of space research that human cells subjected to microgravity reveal a number of alterations in structure and function, including changes in proliferation, but also changes in cytokine production, or in protein kinase C distribution, as well as an elevation of programmed cell death.

To find the reasons for the altered immune response, researchers examined isolated lymphocytes during the first Spacelab missions and focused on the proliferation of these cells (Cogoli et al. 1979). In other studies, they detected an impairment of the response of lymphocytes to mitogenic stimulation (Cogoli et al. 1984; Cogoli and Tschopp 1985).

In addition, signal transduction processes for T-cell activation were disturbed (Cogoli-Greuter 1998; Cogoli-Greuter et al. 2004). In parallel, microgravity induced an increase of apoptosis and enhanced apoptosis-associated Fas/APO-1 proteins in lymphocytes (Jurkat cells) (Lewis et al. 1998).

Maccarrone et al. (2003) demonstrated the induction of 5-lipoxygenase activity and cytochrome c release into the cytosol of human lymphocytes, a result obtained under simulated microgravity conditions and later proven in space on the ISS (Battista et al. 2012). In addition, apoptosis was accompanied by an imbalance of interleukin-2 (IL-2) and interferon-γ (INF-γ), as well as anti- and proapoptotic cytokines (Gasperi et al. 2014). 5-LOX inhibition reduced apoptotic death, restored the initial IL-2/INF-γ ratio and reverted μ-calpain activation induced by simulated microgravity (Gasperi et al. 2014).

In space and on Earth the activation of human T lymphocytes is reduced. Furthermore, human leukocytes exerted changes in the protein kinase C (PKC) distribution (Hatton et al. 1999) and in the IL-2 and IL-2-R-alpha expression (Galleri et al. 2002). In addition, protein kinase A (PKA) is involved in sensing gravity (Boonyaratanakornkit et al. 2005).

In the spaceflight experiment LEUKIN, the investigators found that the transcription of immediate early genes is inhibited in T cells activated in microgravity and that disrupted activation of Rel/NF-κB, CREB1 and SRF transcription factors is involved (Chang et al. 2012).

It had been demonstrated that human T lymphocytes showed a differential inhibition of transcription factor activation in modelled microgravity created by clinorotation (Morrow 2006). AP-1 activation was blocked by clinorotation, whereas NFAT dephosphorylation occurred. Clinorotation inhibits the activation of cellular signaling (Morrow 2006). Another study investigating non-activated human T lymphocytes during a parabolic flight mission showed a downregulation of CD3 and IL-2R surface receptor after 20 s (Tauber et al. 2015). The authors assume that a gravity condition of 1*g* is required for the expression of key surface receptors and appropriate regulation of signal molecules in T lymphocytes (Tauber et al. 2015). These data show that several transcription factors play a role in sensing gravity in human lymphocytes.

Adrian et al. (2013) investigated NR8383 rat alveolar macrophages under altered gravity conditions obtained by parabolic flight maneuvers and clinorotation (2D–clinostat) and focused on the oxidative burst reaction in macrophages, which is a key element in the innate immune response and cellular signaling processes. Their data showed that gravity-sensitive steps are located both in the first activation pathways and in the final oxidative burst reaction. This could be explained by the role of cytoskeletal dynamics in the assembly and function of the NADPH oxidase complex (Adrian et al. 2013).

In CD3/CD28-stimulated primary human T cells, the p21 mRNA expression increased 4.1-fold after 20 s in real microgravity during a parabola in primary CD4+ T cells and 2.9-fold in Jurkat T cells, compared with 1g in-flight controls after CD3/CD28 stimulation. The histone acetyltransferase (HAT) inhibitor curcumin was able to abrogate microgravity-induced p21 mRNA expression, whereas its expression was enhanced by a histone deacetylase (HDAC) inhibitor. The authors supposed that cell cycle progression in human T lymphocytes requires Earth gravity and that the disturbed expression of cell cycle regulatory proteins could contribute to the downregulated immune system of humans in space (Thiel et al. 2012).

5.4 Vascular Cells in Space

Endothelial cells are important for the integrity of the vascular wall. They form the inner layer of blood vessels throughout the whole organism and serve as an anticoagulant barrier between blood and the vessel wall. It is a unique multifunctional cell with important basal and inducible metabolic and synthetic functions (Infanger et al. 2006). It is known that modulation of the endothelial cell function can induce cardiovascular problems and other health problems of humans in space. Endothelial cells are important for several biological processes, such as immune regulation, blood coagulation, growth, extracellular matrix synthesis and others. These processes can be disturbed when the cells are exposed to altered gravity conditions.

Endothelial dysfunction of microvascular endothelial cells (MVECs) may contribute to cardiovascular deconditioning occurring in microgravity. Microgravity conditions induced apoptosis in MVECs. The authors found a downregulation of the PI3K/Akt pathway, an elevation of NF-κB and a depolymerization of F-actin (Kang et al. 2011). Moreover, simulated microgravity can trigger angiogenesis in

endothelial cells and induce tube and spheroid formation (Grimm et al. 2009, 2010, 2014; Ma et al. 2014a, b; Aleshcheva et al. 2016).

A recent paper by Shi et al. showed that clinorotation induces eNOS-mediated angiogenesis in HUVEC cells (Shi et al. 2016). In addition, the authors showed a requirement for Cav-1-associated signaling in microgravity-driven angiogenesis. They suggest that Cav-1 is a critical mediator in simulated weightlessness in endothelium-dependent angiogenesis (Shi et al. 2016).

It has been shown that both P2 receptor gene and protein expression in endothelial cells were altered under clinostat exposure. This indicates that P2 receptors might be important players responding to gravity changes in vascular cells (Zhang et al. 2014).

The post-flight microarray analysis of the ISS SPHINX experiment (HUVEC cells in space) revealed 1023 significantly modulated genes (Versari et al. 2013). The thioredoxin-interacting protein was 33-fold increased, and heat-shock proteins 70 and 90 5.6-fold downregulated. Ion channels, mitochondrial oxidative phosphorylation and focal adhesion were widely affected (Versari et al. 2013). The SPHINX investigators demonstrated that the space environment influences signaling pathways, inducing inflammatory responses, changing endothelial behavior and promoting senescence (Versari et al. 2013).

Endothelial cells exposed to short episodes of real microgravity achieved during parabolic flights exhibited changes in the cytoskeleton and a differential gene expression (Grosse et al. 2012a). In addition, this work showed that caveolins, AMPKα1 and integrins are possible gravi-sensitive elements (Grosse et al. 2012a).

Taking the available results together, protein kinases, integrins, caveolin-1, eNOS, P2 receptors and NF-kB might be key players in sensing gravity in endothelial cells.

5.5 Chondrocytes and Bone Cells

A prolonged exposure to microgravity has deleterious effects on human bone and cartilage (Grimm et al. 2016). Crewmembers suffer after a long-term spaceflight from a reduction of cartilage mass due to mechanical unloading (Zayzafoon et al. 2005).

Neocartilage was formed by porcine chondrocytes cultured in microgravity during the spaceflight 7S (Cervantes mission) on the ISS (Stamenkovic et al. 2010). A weaker extracellular matrix staining of ISS neocartilage tissue was detectable. Higher collagen II/I expression ratios were observed in ISS samples than in control tissue. In addition, there was a lower cell density in ISS neocartilage, which was significantly reduced compared with the normal-gravity neocartilage tissues.

Recent results from ten astronauts who spent more than 5 months in space showed that the cartilage extracellular matrix is sensitive to prolonged exposure to microgravity. This is supported by changes in serum molecular biomarker levels of cartilage turnover. A reduced mechanical loading through microgravity seems to initiate catabolic processes (Niehoff et al. 2016).

Shortterm microgravity during parabolic flight maneuvers had no damaging effects on human chondrocytes. The viability of the cells was normal during the parabolic flight, and a clearly elevated expression of anti-apoptotic genes was detectable after 31st parabolas (Wehland et al. 2015).

A similar result was obtained when human chondrocytes were cultured on the random positioning machine. No signs of apoptosis could be detected (Ulbrich et al. 2010).

Shortterm studies of human chondrocytes exposed to the RPM demonstrated early cytoskeletal changes within 30 min (Aleshcheva et al. 2013). No cytoskeletal changes in chondrocytes were detectable after the first parabola, but they appeared later (vimentin, tubulin, cytokeratin) after the 31st parabola of a parabolic flight, although F-actin remained unaltered (Aleshcheva et al. 2015).

Taking these results for chondrocytes together, chondrocytes exposed to microgravity exhibit only moderate changes of the cytoskeleton. After RPM exposure they change their extracellular matrix production behaviour while they rearrange their cytoskeletal proteins prior to forming three-dimensional aggregates (Fig. 5.4). No signs of programmed cell death were detectable.

A longterm stay in space without available countermeasures enormously affects the bone health of astronauts (Grimm et al. 2016). Bone loss, osteoporosis and bone fractures can occur. One of the mechanisms through which space ventures depress bone formation is through effects on the Wnt/β-catenin signaling pathway. Recent studies have shown that simulated microgravity conditions increase the expression of sclerostin and Dkk-1 in osteocytes (Yang et al. 2015). Wnt-signaling elevates osteoblastic cell differentiation and bone formation, but also inhibits bone resorption. It induces blocking of the receptor activator of nuclear factor-κB-ligand (RANKL)/RANK interaction (Jackson et al. 2005).

There is evidence that although the exact mechanisms are not known, it is possible that sclerostin plays a key role in skeletal adaptation to mechanical forces. Spatz

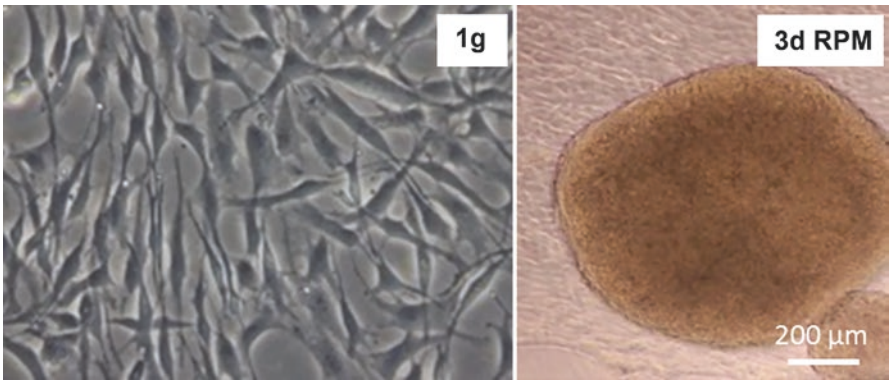

Fig. 5.4 Human chondrocytes (Provitro, Berlin, Germany) cultured under 1*g*–control conditions grew as a 2D monolayer (*left picture*). When they were exposed to the RPM they grew in the form of 3D aggregates and as a 2D monolayer (*right picture*). Phase contrast microscopy was performed by using a Leica microscope (Microsystems GmbH, Wetzlar, Germany). Pictures were taken with a Canon EOS 550D (Canon GmbH, Krefeld, Germany)

et al. investigated the expression of sclerostin in osteocytes *in vitro* and showed that sclerostin is upregulated by mechanical unloading (Spatz et al. 2015). This suggests that mechanical loading regulates intrinsic osteocyte responses (Spatz et al. 2015).

A 5-day spaceflight resulted in an increase in bone resorption by osteoclasts as well as a decrease in osteoblast cellular integrity (Nabavi et al. 2011). Osteoblasts exposed to microgravity exhibited alterations of the microtubules, changes in focal adhesions, and thinner cortical actin and stress fibres (Nabavi et al. 2011).

Real microgravity induces alterations of the cytoskeleton and focal adhesions in bone cells. These are two major mechanosensitive structures. The cytoskeleton responds to changes in the mechanical environment because it is connected to the extracellular matrix through focal adhesions. Exposure of osteoblasts to microgravity impairs their cytoskeleton stability and reduces cellular tension, as well as focal adhesion formation and stability (Hughes-Fulford 2003). Kumei et al. demonstrated that microgravity influences cell adhesion, the cytoskeleton and extracellular matrix proteins of rat osteoblasts cultured in space. Osteopontin and tubulin gene expression levels were downregulated (Kumei et al. 2006).

After a 48 h-clinostat exposure, actin filaments of human osteosarcoma MG63 cells depolymerized, became thinner, and showed a dispersed distribution and disorder, especially in the cytoplasm (Dai et al. 2013).

Summarizing these findings on bone, microgravity clearly influences the cytoskeleton and extracellular matrix proteins in bone cells.

5.6 Cancer Cells Cultured in Microgravity

Different types of cancer cells exposed to microgravity exhibited specific alterations of the cytoskeleton. Human breast cancer cells exposed to real microgravity conditions in space revealed alterations in their microtubules as well as changes in the perinuclear cytokeratin network (Vassy et al. 2001).

These cytoskeletal changes are detectable early. After the first parabola during a parabolic flight, alterations of F-actin were found in follicular thyroid cancer cells (ML-1 cell line) (Ulbrich et al. 2011) and also observed in endothelial cells (EAhy926 cell line) (Grosse et al. 2012a). These findings were proven by the FLUMIAS (Fluorescence Microscopy Analysis System) experiment under microgravity (Fig. 5.5). During the TEXUS-52 sounding rocket flight, the FLUMIAS microscope revealed similar changes in the F-actin network to those observed after RPM-exposure (Corydon et al. 2016b).

When cancer cells were cultured under microgravity conditions, they started to grow three-dimensionally within 24 h, depending on the cell type. DU-145 human prostate carcinoma cells were cultured on a HARV (high-aspect rotating-wall vessel). The authors found that HARV cultivation induced a 3D organoid-like growth type, which was less aggressive, slower growing, less proliferative, more differentiated and a less pliant cell method (Clejan et al. 2001). A similar finding that microgravity induces a phenotype switch to a less aggressive one was detected for

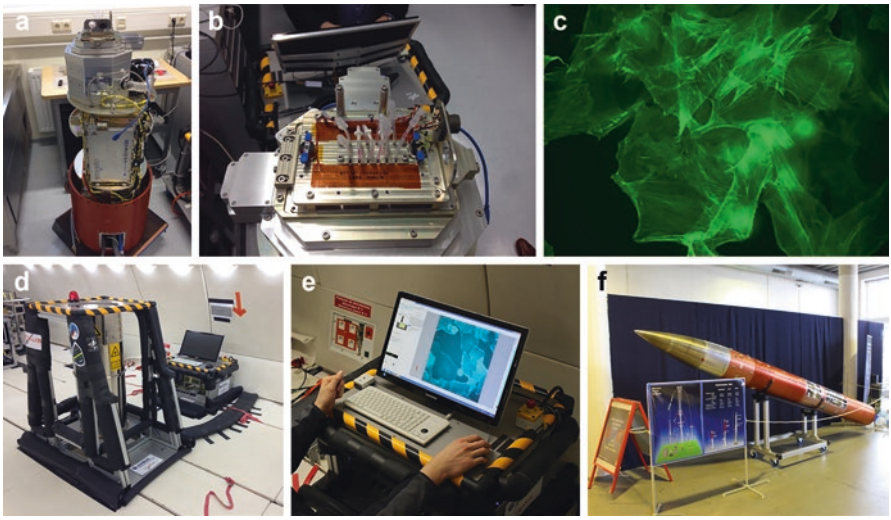

Fig. 5.5 (**a**) Confocal laser scanning microscope (CLSM); (**b**) cross table with observation unit, cells on a slide are fixed on it; (**c**) picture of transfected FTC-133 thyroid cells, the green fluorescence enables the observation of the cytoskeleton (F-Actin); (**d**) CLSM with housing for the testing during the 24th DLR parabolic flight campaign (02.2014); (**e**) picture of FTC-133 cells in the parabolic flight plane; (**f**) TEXUS rocket payload for exhibitions

low-differentiated follicular thyroid cancer cells, which were investigated during the Sino-German space mission Shenzhou-8 in real microgravity (Ma et al. 2014b).

Moreover, 3D growth and the formation of 3D spheroids were confirmed in space (Pietsch et al. 2013). A scaffold-free formation of extraordinarily large 3D aggregates by thyroid cancer cells with altered expression of EGF and CTGF genes was detectable in space. The formation of 3D spheroids had already been demonstrated by exposing a variety of cancer cells to simulated microgravity conditions using a NASA rotary cell culture system (Ingram et al. 1997). The expression of the cell adhesion molecules CD44 and E cadherin was upregulated in the 3D constructs (Ingram et al. 1997).

Follicular thyroid cancer cells investigated on the RPM started to grow in the form of two phenotypes: first as a 2D monolayer and second as a 3D spheroid within 24 h (Grimm et al. 2002; Grosse et al. 2012b; Warnke et al. 2014; Svejgaard et al. 2015; Kopp et al. 2015). Cells grown on the RPM for 24 h exhibited an increase in the NF-κB p65 protein and apoptosis compared to 1g controls, a result also found earlier in endothelial cells. The signaling elements IL-6, IL-8, OPN, TLN1 and CTGF are involved with NF-κB p65 in RPM-dependent thyroid carcinoma cell spheroid formation (Grosse et al. 2012b). In addition, a device comparison study (RPM and 2D clinostat) demonstrated changes in the regulation of *CTGF* and *CAV1* appearing in a comparable manner on both machines. Both factors seem to play a role in 3D formation (Warnke et al. 2014). IL-6 and IL-8 application to the medium of ML-1 cancer cells has shown a direct influence on 3D formation (Svejgaard et al.

2015). In a longterm study, FTC-133 low-differentiated follicular thyroid cancer cells and normal thyrocytes were cultured on the RPM for 14 days (Kopp et al. 2015). Significant differences between normal and cancer cells were found concerning the gene expression of *NGAL, VEGFA*, *OPN*, *IL6* and *IL17* and the secretion of VEGFA, IL-17 and IL-6 (Kopp et al. 2015). These data suggest their involvement in 3D formation of thyroid cells after RPM exposure.

Taking these data together, cancer cells studied in real or simulated microgravity show early cytoskeletal changes, apoptosis and 3D growth. Several factors are known to be involved in the aggregation process and phenotype switch of the investigated cells. These are the growth factors CTGF, EGF and VEGF, the cytokines IL-6, IL-8 and IL-17 and NF-kB.

5.7 Hypothesis on How Gravity Is Perceived by Human Cells: The Tensegrity Model—How Unspecialized Human Cells Might Sense Gravity

As described, human cells *in vitro* can react to mechanical unloading in different ways; however, the question arises as to how they are able to sense the rather weak changes in force.

Ever since Rijken et al. found significant alterations of the cytoskeleton in human A431 cells during a TEXUS flight in 1991 (Rijken et al. 1991a, b), the cytoskeleton has been a hot candidate for transmitting mechanical unloading from the cells' environment.

How the cells manage to transform the mechanical signal into a biochemical one is still today a current topic under investigation. However, an increasing yield of data supports the tensegrity model hypothesis, proposed by Ingber (1999).

The tensegrity model claims that cells are hardwired by the different parts of the cytoskeleton, which are connected to discrete cell adhesions. According to this model, cells are spanned open and are under continuous tension. The adhesion points are connected to the extracellular matrix. In sum, there is a balance of force between the extracellular matrix, adhesion points and the cytoskeleton in normal gravity conditions. Therefore, an imbalance of adhesion and cytoskeleton would result in a change of cell shape and have a direct impact on signaling cascades and downstream transcription events (Ingber 1999).

This theory is supported by various findings of cytoskeletal changes in different cell types after short-term exposure to real and simulated microgravity (Vorselen et al. 2014). Fixation of thyroid cancer cells and endothelial cells as well as chondrocytes after 22 s of microgravity revealed that actin fibres and/or microtubules were localized close to the nucleus while losing their distinct polarization (Grosse et al. 2012a; Aleshcheva et al. 2015; Ulbrich et al. 2011). These findings are in concert with significant gene expression changes after 22 s of real microgravity.

However, artefact building during fixation could not be neglected until Corydon et al. first investigated life-act GFP marked thyroid cancer cells during a parabolic

flight campaign and the TEXUS 52 campaign in Kiruna, Sweden (Corydon et al. 2016b). Live imaging of the cells during microgravity revealed an instant rearrangement of actin filaments and a rapid change of cell shape.

These findings are in concert with gene expression changes in cytoskeletal genes and proteins, which are involved in proliferation and differentiation. Finally, these experiments further increased the evidence of a direct correlation between the cytoskeletal rearrangements upon microgravity and transcription alterations and strongly suggest that the interaction between the extracellular matrix, adhesion and connected cytoskeleton is a major part in gravi-sensing non-specialized human cells.

References

Adrian A, Schoppmann K, Sromicki J, Brungs S, von der Wiesche M, Hock B, et al. (2013) The oxidative burst reaction in mammalian cells depends on gravity. Cell Commun Signal 11:98

Aleshcheva G, Sahana J, Ma X, Hauslage J, Hemmersbach R, Egli M et al. (2013) Changes in morphology, gene expression and protein content in chondrocytes cultured on a random positioning machine. PLoS One 8:e79057

Aleshcheva G, Wehland M, Sahana J, Bauer J, Corydon TJ, Hemmersbach R et al. (2015) Moderate alterations of the cytoskeleton in human chondrocytes after short-term microgravity produced by parabolic flight maneuvers could be prevented by up-regulation of BMP-2 and SOX-9. FASEB J 29:2303–2314

Aleshcheva G, Bauer J, Hemmersbach R, Slumstrup L, Wehland M, Infanger M et al. (2016) Scaffold-free tissue formation under real and simulated microgravity conditions. Basic Clin Pharmacol Toxicol 119(suppl 3):26–33

Battista N, Meloni MA, Bari M, Mastrangelo N, Galleri G, Rapino C et al. (2012) 5-Lipoxygenase-dependent apoptosis of human lymphocytes in the International Space Station: data from the ROALD experiment. FASEB J 26:1791–1798

Boonyaratanakornkit JB, Cogoli A, Li CF, Schopper T, Pippia P, Galleri G et al. (2005) Key gravity-sensitive signaling pathways drive T cell activation. FASEB J 19:2020–2022

Chang TT, Walther I, Li CF, Boonyaratanakornkit J, Galleri G, Meloni MA et al. (2012) The Rel/NF-kappaB pathway and transcription of immediate early genes in T cell activation are inhibited by microgravity. J Leukoc Biol 92:1133–1145

Clejan S, O'Connor K, Rosensweig N (2001) Tri-dimensional prostate cell cultures in simulated microgravity and induced changes in lipid second messengers and signal transduction. J Cell Mol Med 5:60–73

Cogoli A, Tschopp A (1985) Lymphocyte reactivity during spaceflight. Immunol Today 6:1–4

Cogoli A, Valluchi-Morf M, Bohringer HR, Vanni MR, Muller M (1979) Effect of gravity on lymphocyte proliferation. Life Sci Space Res 17:219–224

Cogoli A, Tschopp A, Fuchs-Bislin P (1984) Cell sensitivity to gravity. Science 225:228–230

Cogoli-Greuter M (1998) Influence of microgravity on mitogen binding, motility and cytoskeleton patterns of T lymphocytes and Jurkat cells-experiments on sounding rockets. Jpn J Aerospace Environ Med 35:27–39

Cogoli-Greuter M, Lovis P, Vadrucci S (2004) Signal transduction in T cells: an overview. J Gravit Physiol 11:53–56

Corydon TJ, Kopp S, Wehland M, Braun M, Schutte A, Mayer T et al (2016b) Alterations of the cytoskeleton in human cells in space proved by life-cell imaging. Sci Rep 6:20043

Corydon TJ, Mann V, Slumstrup L, Kopp S, Sahana J, Askou AL et al (2016a) Reduced expression of cytoskeletal and extracellular matrix genes in human adult retinal pigment epithelium cells exposed to simulated microgravity. Cell Physiol Biochem 40:1–17

Dai Z, Wu F, Chen J, Xu H, Wang H, Guo F et al. (2013) Actin microfilament mediates osteoblast Cbfa1 responsiveness to BMP2 under simulated microgravity. PLoS One 8:e63661

Galleri G, Meloni MA, Camboni MG, Deligios M, Cogoli A, Pippia P (2002) Signal transduction in T lymphocites under simulated microgravity conditions: involvement of PKC isoforms. J Gravit Physiol 9:289–290

Gasperi V, Rapino C, Battista N, Bari M, Mastrangelo N, Angeletti S et al. (2014) A functional interplay between 5-lipoxygenase and mu-calpain affects survival and cytokine profile of human Jurkat T lymphocyte exposed to simulated microgravity. Biomed Res Int 2014:782390

Grimm D, Bauer J, Kossmehl P, Shakibaei M, Schoberger J, Pickenhahn H et al. (2002) Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. FASEB J 16(6):604–606

Grimm D, Infanger M, Westphal K, Ulbrich C, Pietsch J, Kossmehl P et al. (2009) A delayed type of three-dimensional growth of human endothelial cells under simulated weightlessness. Tissue Eng Part A 15:2267–2275

Grimm D, Bauer J, Ulbrich C, Westphal K, Wehland M, Infanger M et al. (2010) Different responsiveness of endothelial cells to vascular endothelial growth factor and basic v growth factor added to culture media under gravity and simulated microgravity. Tissue Eng Part A 16:1559–1573

Grimm D, Wise P, Lebert M, Richter P, Baatout S (2011) How and why does the proteome respond to microgravity? Expert Rev Proteomics 8:13–27

Grimm D, Wehland M, Pietsch J, Aleshcheva G, Wise P, van Loon J et al. (2014) Growing tissues in real and simulated microgravity: new methods for tissue engineering. Tissue Eng Part B Rev 20:555–566

Grimm D, Grosse J, Wehland M, Mann V, Reseland JE, Sundaresan A, Corydon TJ (2016) The impact of microgravity on bone in humans. Bone 87:44–56

Grosse J, Wehland M, Pietsch J, Ma X, Ulbrich C, Schulz H et al. (2012a) Short-term weightlessness produced by parabolic flight maneuvers altered gene expression patterns in human endothelial cells. FASEB J 26:639–655

Grosse J, Wehland M, Pietsch J, Schulz H, Saar K, Hubner N et al. (2012b) Gravity-sensitive signaling drives 3-dimensional formation of multicellular thyroid cancer spheroids. FASEB J 26:5124–5140

Hatton JP, Gaubert F, Lewis ML, Darsel Y, Ohlmann P, Cazenave JP et al. (1999) The kinetics of translocation and cellular quantity of protein kinase C in human leukocytes are modified during spaceflight. FASEB J 13(suppl):S23–S33

Hughes-Fulford M (2003) Function of the cytoskeleton in gravisensing during spaceflight. Adv Space Res 32:1585–1593

Infanger M, Kossmehl P, Shakibaei M, Baatout S, Witzing A, Grosse J et al. (2006) Induction of three-dimensional assembly and increase in apoptosis of human endothelial cells by simulated microgravity: impact of vascular endothelial growth factor. Apoptosis 11:749–764

Ingber D (1999) How cells (might) sense microgravity. FASEB J 13(suppl):S3–15

Ingram M, Techy GB, Saroufeem R, Yazan O, Narayan KS, Goodwin TJ et al. (1997) Three-dimensional growth patterns of various human tumor cell lines in simulated microgravity of a NASA bioreactor. In Vitro Cell Dev Biol Anim 33:459–466

Jackson A, Vayssiere B, Garcia T, Newell W, Baron R, Roman-Roman S et al. (2005) Gene array analysis of Wnt-regulated genes in C3H10T1/2 cells. Bone 36:585–598

Kang CY, Zou L, Yuan M, Wang Y, Li TZ, Zhang Y et al. (2011) Impact of simulated microgravity on microvascular endothelial cell apoptosis. Eur J Appl Physiol 111:2131–2138

Kopp S, Warnke E, Wehland M, Aleshcheva G, Magnusson NE, Hemmersbach R et al. (2015) Mechanisms of three-dimensional growth of thyroid cells during long-term simulated microgravity. Sci Rep 5:16691

Kumei Y, Morita S, Katano H, Akiyama H, Hirano M, Oyha K et al. (2006) Microgravity signal ensnarls cell adhesion, cytoskeleton, and matrix proteins of rat osteoblasts: osteopontin, CD44, osteonectin, and alpha-tubulin. Ann N Y Acad Sci 1090:311–317

Lewis ML, Reynolds JL, Cubano LA, Hatton JP, Lawless BD, Piepmeier EH (1998) Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). FASEB J 12:1007–1018

Ma X, Sickmann A, Pietsch J, Wildgruber R, Weber G, Infanger M et al. (2014a) Proteomic differences between microvascular endothelial cells and the EA.hy926 cell line forming three-dimensional structures. Proteomics 14:689–698

Ma X, Pietsch J, Wehland M, Schulz H, Saar K, Hubner N et al. (2014b) Differential gene expression profile and altered cytokine secretion of thyroid cancer cells in space. FASEB J 28:813–835

Maccarrone M, Battista N, Meloni M, Bari M, Galleri G, Pippia P et al. (2003) Creating conditions similar to those that occur during exposure of cells to microgravity induces apoptosis in human lymphocytes by 5-lipoxygenase-mediated mitochondrial uncoupling and cytochrome c release. J Leukoc Biol 73:472–481

Mader TH, Gibson CR, Pass AF, Kramer LA, Lee AG, Fogarty J et al. (2011) Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. Ophthalmology 118:2058–2069

Mader TH, Gibson CR, Lee AG (2016) Choroidal folds in astronauts. Invest Ophthalmol Vis Sci 57:592

Morrow MA (2006) Clinorotation differentially inhibits T-lymphocyte transcription factor activation. In Vitro Cell Dev Biol Anim 42:153–158

Nabavi N, Khandani A, Camirand A, Harrison RE (2011) Effects of microgravity on osteoclast bone resorption and osteoblast cytoskeletal organization and adhesion. Bone 49:965–974

Niehoff A, Brüggemann GP, Zaucke F, Eckstein F, Bloch W, Mündermann A et al. (2016) Long-duration space flight and cartilage adaptation: first results on changes in tissue metabolism. Osteoarthr Cartil 24:S144–S145

Pietsch J, Bauer J, Egli M, Infanger M, Wise P, Ulbrich C et al. (2011) The effects of weightlessness on the human organism and mammalian cells. Curr Mol Med 11:350–364

Pietsch J, Ma X, Wehland M, Aleshcheva G, Schwarzwalder A, Segerer J et al. (2013) Spheroid formation of human thyroid cancer cells in an automated culturing system during the Shenzhou-8 Space mission. Biomaterials 34:7694–7705

Rijken PJ, de Groot RP, Briegleb W, Kruijer W, Verkleij AJ, Boonstra J et al. (1991a) Epidermal growth factor-induced cell rounding is sensitive to simulated microgravity. Aviat Space Environ Med 62:32–36

Rijken PJ, Hage WJ, van Bergen en Henegouwen PM, Verkleij AJ, Boonstra J (1991b) Epidermal growth factor induces rapid reorganization of the actin microfilament system in human A431 cells. J Cell Sci 100(Pt 3):491–499

Shi F, Zhao TZ, Wang YC, Cao XS, Yang CB, Gao Y et al. (2016) The impact of simulated weightlessness on endothelium-dependent angiogenesis and the role of caveolae/caveolin-1. Cell Physiol Biochem 38:502–513

Spatz JM, Wein MN, Gooi JH, Qu Y, Garr JL, Liu S et al. (2015) The Wnt inhibitor sclerostin is up-regulated by mechanical unloading in osteocytes in vitro. J Biol Chem 290:16744–16758

Stamenkovic V, Keller G, Nesic D, Cogoli A, Grogan SP (2010) Neocartilage formation in 1 g, simulated, and microgravity environments: implications for tissue engineering. Tissue Eng Part A 16:1729–1736

Svejgaard B, Wehland M, Ma X, Kopp S, Sahana J, Warnke E et al. (2015) Common effects on cancer cells exerted by a random positioning machine and a 2d clinostat. PLoS One 10:e0135157

Tauber S, Hauschild S, Paulsen K, Gutewort A, Raig C, Hurlimann E et al. (2015) Signal transduction in primary human T lymphocytes in altered gravity during parabolic flight and clinostat experiments. Cell Physiol Biochem 35:1034–1051

Thiel CS, Paulsen K, Bradacs G, Lust K, Tauber S, Dumrese C et al. (2012) Rapid alterations of cell cycle control proteins in human T lymphocytes in microgravity. Cell Commun Signal 10:1

Ulbrich C, Westphal K, Pietsch J, Winkler HD, Leder A, Bauer J et al. (2010) Characterization of human chondrocytes exposed to simulated microgravity. Cell Physiol Biochem 25:551–560

Ulbrich C, Pietsch J, Grosse J, Wehland M, Schulz H, Saar K et al. (2011) Differential gene regulation under altered gravity conditions in follicular thyroid cancer cells: relationship between the extracellular matrix and the cytoskeleton. Cell Physiol Biochem 28:185–198

Vassy J, Portet S, Beil M, Millot G, Fauvel-Lafeve F, Karniguian A et al. (2001) The effect of weightlessness on cytoskeleton architecture and proliferation of human breast cancer cell line MCF-7. FASEB J 15:1104–1106

Versari S, Longinotti G, Barenghi L, Maier JA, Bradamante S (2013) The challenging environment on board the International Space Station affects endothelial cell function by triggering oxidative stress through thioredoxin interacting protein overexpression: the ESA-SPHINX experiment. FASEB J 27:4466–4475

Vorselen D, Roos WH, MacKintosh FC, Wuite GJ, van Loon JJ (2014) The role of the cytoskeleton in sensing changes in gravity by nonspecialized cells. FASEB J 28:536–547

Warnke E, Pietsch J, Wehland M, Bauer J, Infanger M, Gorog M et al. (2014) Spheroid formation of human thyroid cancer cells under simulated microgravity: a possible role of CTGF and CAV1. Cell Commun Signal 12:32

Wehland M, Aleshcheva G, Schulz H, Saar K, Hubner N, Hemmersbach Ret al. (2015) Differential gene expression of human chondrocytes cultured under short-term altered gravity conditions during parabolic flight maneuvers. Cell Commun Signal 13:18

White RJ, Averner M (2001) Humans in space. Nature 409:1115–1118

Yang X, Sun L-W, Liang M, Wang X-N, Fan Y-B (2015) The response of wnt/ß-catenin signaling pathway in osteocytes under simulated microgravity. Microgravity Sci Tech 27:473–483

Zayzafoon M, Meyers VE, McDonald JM (2005) Microgravity: the immune response and bone. Immunol Rev 208:267–280

Zhang Y, Lau P, Pansky A, Kassack M, Hemmersbach R, Tobiasch E (2014) The influence of simulated microgravity on purinergic signaling is different between individual culture and endothelial and smooth muscle cell coculture. Biomed Res Int 2014:413708

Zwart SR, Gregory JF, Zeisel SH, Gibson CR, Mader TH, Kinchen JM et al. (2016) Genotype, B-vitamin status, and androgens affect spaceflight-induced ophthalmic changes. FASEB J 30:141–148