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SYSTEMATIC REVIEW ARTICLE

Gene Expression in Osteoblasts and Osteoclasts Under Microgravity Conditions: A Systematic Review

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Abstract: ***Background*:** Microgravity (μG) negatively influences bone metabolism by affecting normal osteoblast and osteoclast function. μG effects on bone metabolism has been an extensive field of study in recent years, due to the challenges presented by space flight.

***Methods*:** We systematically reviewed research data from genomic studies performed in real or simulated μG, on osteoblast and osteoclast cells. Our search yielded 50 studies, of which 39 concerned cells of the osteoblast family and 11 osteoclast precursors.

***Results*:** Osteoblastic cells under μG show a decreased differentiation phenotype, proved by diminished expression levels of Alkaline Phosphatase (ALP) and Osteocalcin (OCN) but no apoptosis. Receptor Activator of NF-κB Ligand (RANKL)/ Osteoprotegerine (OPG) ratio is elevated in favor of RANKL in a time-dependent manner, and further RANKL production is caused by upregulation of Interleukin-6 (IL-6) and the inflammation pathway. Extracellular signals and changes in the gravitational environment are perceived by mechanosensitive proteins of the cytoskeleton and converted to intracellular signals through the Mitogen Activated Protein Kinase pathway (MAPK). This is followed by changes in the expression of nuclear transcription factors of the Activator Protein-1 (AP-1) family and in turn of the NF-κB, thus affecting osteoblast differentiation, cell cycle, proliferation and maturation. Pre-osteoclastic cells show increased expression of the marker proteins such as Tryptophan Regulated Attenuation Protein (TRAP), cathepsin K, Matrix Metalloproteinase-9 (MMP-9) under μG conditions and become sensitized to RANKL.

***Conclusion*:** Suppressing the expression of fusion genes such as syncytine-A which acts independently of RANKL, could be possible future therapeutic targets for microgravity side effects.

Keywords: Osteoblasts, osteoclasts, microgravity, gene expression, microarrays, space.

1. INTRODUCTION

All living organisms have evolved under the effect of Earth's inherent gravity of 1g. Since the first manned mission in space, research has focused on the impact of reduced gravitational force in the biological processes of organisms. Astronauts taking part in space missions suffer the effects of gravitational alterations, varying from increased short-term accelerations at launch, to long-term decreased gravity in orbit, known as microgravity (μG)[1]. Among the various systems of the human organism that are affected by altered gravity is the skeletal system. Skeletal unloading due to μG results in 1-2% loss of bone mass per month, mainly in the pelvis and the lower extremities. Microgravity influences bone structure macroscopically and in the cellular level [1-3]. Human bone homeostasis is regulated by the coupled and synergic actions of specialized cells called osteoblasts and osteoclasts [4]. Until recently, scientists could study the influence of microgravity on gene expression, only on small groups of genes per experiment. Advances in engineering, biotechnology and information technology have facilitated the invention of high throughput methods such as the microarray and Next Generation Sequencing (NGS) technologies, which in turn enabled the complete genomic study of cells, in a way that is easily reproducible [5, 6]. Experiments in space flights are scarce mainly because of the high cost of space flights. Systems that simulate microgravitational conditions on earth have been used to perform experiments in cell cultures and organisms using different principles. *Hind-*

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[1] https://en.wikipedia.org/wiki/Micro-g_environment

Limb Suspension (HLS), *Head Down Bed Rest* (HDBR), the NASA-developed *Rotary Cell Culture System* (RCCS), Three Dimension Clinostat (3D Clinostat), *Random Positioning Machine* (RPM), Large Gradient High Magnetic Field (LG-HMF) are the methods most widely used in this field of research [7-9].

2. METHODS

The current study was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements [10]. Two independent reviewers performed the literature search as well as the evaluation of the identified studies to determine the eligibility for inclusion. A third reviewer was included in order to resolve any possible differences derived from the above-mentioned process.

2.1. Data Sources

The studies included in the current systematic review were retrieved from an independent literature search performed in the PubMed and Cochrane databases as well as other sources like Google Scholar and NASA Technical Reports Server. Independent keywords along with their combinations were applied to both databases. The specific keywords were the following: "*microgravity*", "*weightlessness*", "*spaceflight*", "*osteoblast*", "*osteoclast*" and "*shuttle*". The above-mentioned keywords were generated through evaluation of the MeSH (Medical Subject Headings) database. Specifically, the search string applied to PubMed was: osteoblast [Title/Abstract]) OR osteoclast [Title/Abstract]) AND weightlessness [Title/Abstract] OR osteoblast [Title/Abstract]) OR osteoclast [Title/Abstract]) AND microgravity [Title/Abstract] OR osteoblast [Title/Abstract]) OR osteoclast [Title/Abstract]) AND spaceflight [Title/Abstract]. The search string applied to the Cochrane database was: microgravity AND osteoblast OR microgravity AND osteoclast.

2.2. Study Selection Criteria

In order to be included in the current review, a study is needed to adhere to specific inclusion and exclusion criteria. Particularly, a study should have provided data regarding gene expression of cells from the osteoblast or osteoclast cell lines incubated in microgravity or simulated microgravity conditions. Specifically, the study material included the a) MC3T3-E1 osteoblast precursor cell line derived from *Mus musculus* (mouse) calvaria, b) the MG-63 *homo sapiens* (human) bone osteosarcoma cells (fibroblasts), c) human osteoblast cells, d) chicken calvaria osteoblast cells, e) rat pre-osteoblasts, f) 2T3 immortalized murine osteoblast cell line, osteoblasts and osteoclasts derived from pharyngeal bone of medaka fish and g) the RAW 264.7 *mus musculus* (mouse) macrophage cells, murine (mouse) macrophages from bone marrow as well as mature osteoclasts.

As "*microgravity*" and "*simulated microgravity*" was considered the environment of reduced gravitational pull that is present in space shuttles, in orbit around the earth or when performing short parabolic flights and in ground facilities with devices that simulate weightlessness. The latter are the NASA developed Rotary Cell Culture System (RCCS) or Rotating Wall Vessel (RWV), the 2D or 3D Clinostat, the Random Positioning Machine (RPM), the Large Gradient High Magnetic Field (LG-HMF), Hind limb suspension (HLS) and Head Down Bed Rest (HDBR). Prior reviews that focused on a similar subject, were not considered as eligible for inclusion. Moreover, studies involving ancestral bone mesenchymal cells or mature osteocytes were also excluded, as well as studies that included only proteomic data with no adherent genomic data. Articles that the full text was not available were also excluded. Time or country of origin restrictions were not applied during the identification of eligible studies, whereas studies that were published in languages other than English were not considered as eligible for inclusion.

2.3. Data Extraction

Two authors independently reviewed the full texts of all studies that were considered eligible for inclusion and extracted the individual study data. Any discrepancies were resolved by discussion with a third author to reach a final consensus. Specifically, the data extracted included the following: study characteristics (first author, year of publication, study design), organism and cell line characteristics, location and duration of the experiment (ground based or space), method used to simulate microgravity in ground based studies, methodology of gene expression (microarray, RT-PCR, Hs DNA) and type of statistical analysis performed.

3. RESULTS

Our search in databases PubMed and Cochrane produced 176 results and additional 157 from other sources. After duplicates were removed the remaining study number was 257. After detailed screening 50 studies were eligible for qualitative synthesis in the current systematic review. The process of study selection is depicted in Fig. (**1**), which consists of the actual flow diagram we followed.

3.1. Study Characteristics

A total of 50 studies met the predefined criteria and were included in the systematic review. Eleven of 50 referred to osteoclast gene expression in microgravity (Ethiraj *et al.* 2018 [11], Shanmugarajan *et al.* 2017 [12], Sambandam *et al.* 2016 [13], Chatani *et al.* 2016 [14], Chatani *et al.* 2015 [15], Sun *et al.* 2015 [16], Saxena *et al.* 2011 [17], Sambandam *et al.* 2010 [18], Sambandam *et al.* 2014 [19], Tamma *et al.* 2009 [20], Makihira *et al.* 2008 [21]) [11-21] and 39 out of 50 to osteoblast (Wang *et al.* 2016 [22], Sun *et al.* 2015 [23], Hu *et al.* 2015 [24], Makihira *et al.* 2008 [21], Bucaro *et al.* 2007 [25], Hughes *et al.* 2006 [26], Bucaro *et al.* 2004 [27], Saito *et al.* 2003 [28], Ontiveros *et al.* 2003 [29], Kumei *et al.* 2002 [30], Sato *et al.* 1999 [31], Hughes *et al.* 1998 [32], Z Hu *et al.* 2017 [33], Goyden *et al.* 2015 [34], Bikle *et al.* 1994 [35], Qian *et al.* 2009 [36], Shuang *et al.* 2013 [37], Dai *et al.* 2013 [38], Kapitonova *et al.* 2013 [39], Guignandon *et al.* 2014 [40], Kumei *et al.* 1996 [41], Carmeliet *et al.* 1999 [42], Landis *et al.* 2000 [43], Rucci *et al.* 2002 [44], Kumei *et al.* 2003 [45, 46], Nakamura *et al.* 2003 [47], Kumei *et al.* 2004 [48, 49], Kumei *et al.* 2006 [50], Pardo *et al.* 2005 [51], Rucci *et al.* 2007 [52], Patel *et al.*

2007 [53], Kumei *et al.* 2007 [54], Capulli *et al.* 2009 [55], Blaber *et al.* 2013 [56], Rucci *et al.* 2015 [57], Chatani *et al.* 2016 [14], Hu *et al.* 2015 [58]). With regard to the location that the experiments took place 21 were performed in space (shuttles, International Space Station (ISS), rockets) and 39 in ground simulators [59]. There were some comparative studies that duplicated the experiments in space and ground based microgravity models. The duration of microgravity in ground simulations varied from 1-7 days for RCCS, 2D or 3D Clinostat and LG-HMF and from 2-8 weeks for HLS or HDBR where exposure in space was 1-60 days. Detailed characteristics are depicted in Tables **1** and **2**.

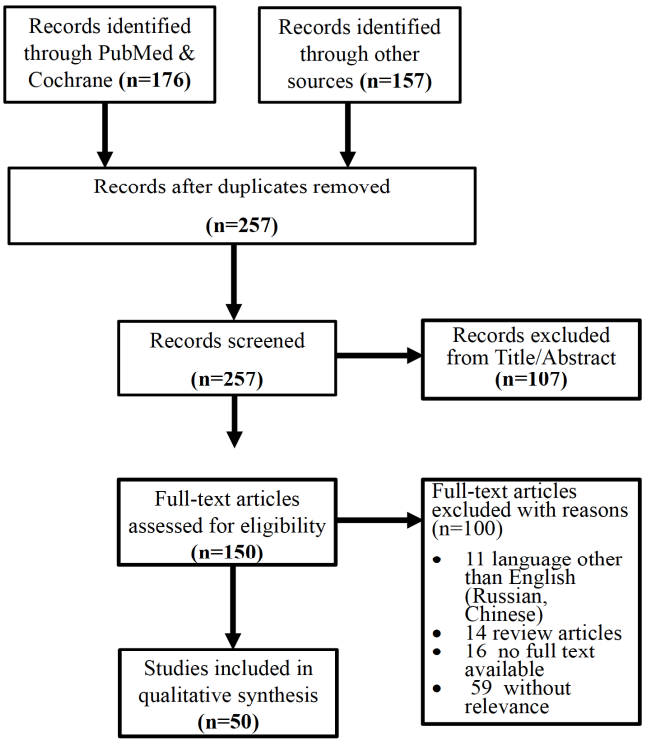

Fig. (1). PRISMA results and literature search workflow.

3.2. Microgravity's Effect on Osteoblast Differentiation and Matrix Mineralization

Twenty of 50 studies included genomic data on the expression of marker genes involved in the process of osteoblastic cell differentiation and matrix mineralization. Runt related transcription factor 1 (Runx2) and Osterix (Ostx) are key factors that are implicated early in the maturation of osteoblasts. Runx2 was downregulated in 8 studies when no difference in expression levels was detected in 2. Furthermore, one study showed a gradual increase in expression from the 3rd day, but overall levels were diminished compared to ground controls. Ostx was also downregulated in one study (minimum on 2nd day) whereas in another that had a 7-day duration a gradual increase was noted up to the 7th day. Interestingly high levels were detected throughout the duration of a study performed in space, that included living organisms but with less statistical significance. RANKL was found upregulated early when exposed to μG, while OPG showed a gradual and slower increase. Maximum expression of RANKL was reached on the 2nd day and osteoprotegerine (OPG) on the 7th day but the results were not significant compared to ground controls. Notably when the ratio of RANKL/OPG was calculated, it reached significant levels, which was a common finding in all three studies. Alkaline phosphatase (ALP) and collagen I (CollI) mRNA levels manifested a somewhat diversity. Four short-duration earth studies show downregulation of ALP when five others, conducted on earth simulators as well as in space, conclude that ALP is upregulated but in lower levels than on earth gravity. One study that lasted a day showed no difference in ALP expression. CollI is expressed in a time-dependent manner with maximum levels on the 7th day of exposure. Osteocalcin (OCN), a protein implicated in matrix mineralization was upregulated in four studies, showed no difference in two and was downregulated in four. Details are depicted in Table **3**.

3.3. Microgravity's Effect on Osteoblast Cytoskeleton, Growth, Proliferation and Apoptosis

A great number of proteins and molecules are involved in osteoblast mechanosensation and cell survival metabolic pathways. The RNA expression of cytoskeleton related genes was found increased for tallin, paxillin, supervillin, Wiskott-Aldrich Syndrome Protein Family Member 2 (ASF2), WAS Interacting Protein family member 1(WIPF1) while actin, a-tubulin and heat shock protein 73(HSP73) were decreased (Table **4**). Apoptotic molecules like Bax and p53 showed upregulation that was countered by a concomitant increase in the anti-apoptotic Bcl, p21 and X-linked inhibitor of apoptosis(XIAP). Transcription factors c-fos and c-jun, that respond early in stressful stimuli and convert extracellular signals into changes in gene expression in the nucleus, where upregulated (Table **5**). A number of cytokines and growth factors produced by osteoblast contribute to regulating bone homeostasis. Prostaglandin E2 (PGE-2) which has a positive effect on bone formation was upregulated in μG. Interestingly cyclooxygenase 2 (cox2), an enzyme that normally increases PGE-2 production by a feedback mechanism, was found downregulated. Interleukin-6 (IL-6) that supports and enhances osteoclastic activity was upregulated in three studies and downregulated in one (Table **6**).

3.4. Microgravity's Effect on Osteoclasts

The compilation of eleven studies concerning osteoclast genomic activity in microgravity conditions revealed upregulation in genes involved in differentiation, maturation and metabolic function. Specifically c-fos, jun-b-like and ddit4, that are involved in nuclear and mitochondrial signaling, where overexpressed as were fusion and proliferation molecules like syncytin-A, dendrocyte expressed transmembrane protein(DCSTAMP), osteoclast stimulator transmembrane protein(OCSTAMP), TNF receptor associated factor(TRAF) and TNF related apoptosis-inducing factor(TRAIL). Pre-osteoclastic cells showed increased expression of TRAP, cathepsin K and matrix metalloproteinases (MMPs) indicating osteoclast maturation. Adhesion protein β-integrin and receptor activator of NF-κB (RANK) levels were increased in response to RANKL, as well as autophagy proteins Atg5 and LC3 independent of RANKL (Table **7**).

Table 1. Study characteristics for osteoblastic cell lines.

References	Study Design					Genomic Data
	Organism	Cell Type	Type of μG	Location	Duration	
Hu *et al*. 2017 [33]	mouse	MC3T3-E1	RWV	EARTH	3h	Microarray(Agilent)
Wang 2016 *et al*. [22]	mouse	MC3T3-E1	clinorotation	EARTH	2d	Total RNA-cDNA-qRT-PCR
Chatani *et al*. 2016 [14]	medaka fish	OB cells	ISS	SPACE	1-8d	HiSeq
Sun *et al*. 2015 [16]	mouse	MC3T3-E1	clinorotation	EARTH	2d	Total RNA-cDNA-qRT-PCR
Sun *et al*. 2015 [23]	mouse	MC3T3-E1	clinorotation	EARTH	2d, 3d	qPCR
Goyden *et al*. 2015 [34]	mouse	MC3T3-E1	RCCS	EARTH	7d	Total RNA-cDNA-qRT-PCR
Rucci *et al*. 2015 [57]	human	OB cells	HDBR	EARTH	14d	Total RNA-cDNA
	mouse	OB cells	HLS	EARTH	21d	Total RNA-cDNA
	mouse	OB cells	BOTOx(quad, soleus, gastro, Plantaris)	EARTH	21d	Total RNA-cDNA
Hu *et al*. 2015 [58]	rat	OB cells(femur)	HLU	EARTH	3w	Microarray(Agilent)
	-	prOB	2D-RWV	EARTH	2d	Microarray(Agilent)
Hu Li *et al*. 2015 [24]	Mouse	MC3T3-E1	3D-RPM	EARTH	1d	RT-PCR & Real time PCR
Guignandon 2014 *et al*. [40]	human	MG-63	FOTON-M3	SPACE	69h	Total RNa & real time PCR
Dai *et al*. 2013 [38]	human	OSE-MG-63	clinostat	EARTH	2d	Total RNA-qPCR
Blaber *et al*. 2013 [56]	mouse	OB cells	STS-131	SPACE	15d	qRT-PCR arrays (Qiagen)
		prOB	RWV	EARTH	2d	qRT-PCR arrays (Qiagen)
Kapitonova *et al*. 2013 [39]	human	OB cells	ISS	SPACE	10d	Total RNA-cDNA-RT PCR(Qiagen)
Shuang *et al*. 2012 [37]	mouse	femur	HLS	EARTH	4w & 8w	qRT -PCR
	mouse	2T3	RCCS	EARTH	2d	qRT -PCR
Qian *et al*. 2009 [36]	human	MG-63	LG-HMF	EARTH	1d	Microarray(Affymetrix)
Capulli *et al*. 2009 [55]	mouse	OB cells	RWV	EARTH	5d	Microarray(Affymetrix)-real time PCR
Makihira *et al*. 2008 [21]	mouse	MC3T3-E1	RPM	EARTH	3d & 7d	qRT-PCR
Kumei *et al*. 2007 [54]	rat	OB cells	space shuttle	SPACE	4d & 5d	RT-PCR
Bucaro *et al*. 2007 [25]	mouse	MC3T3-E1	HARV(CLINOSTAT)	EARTH	1d & 5d & 14d	RT-PCR
Rucci *et al*. 2007 [52]	mouse	OB cells	RWV	EARTH	1d	RT-PCR
Patel *et al*. 2007 [53]	mouse	2T3	RWV	EARTH	3d	Microarray(Affymetrix)
Hughes *et al*. 2006 [26]	mouse	MC3T3-E1	STS-76	SPACE	24h	RT-PCR
Kumei *et al*. 2006 [50]	rat	OB cells	space shuttle	SPACE	4d & 5d	RT-PCR

(Table 1) contd….

References	Study Design					Genomic Data
	Organism	Cell Type	Type of μG	Location	Duration	
Pardo *et al.* 2005 [51]	mouse	2T3	RPM	EARTH	3d	Microarray(Amersham)
Bucaro *et al.* 2004 [27]	mouse	MC3T3-E1	RCCS	EARTH	5d	RT-PCR
Kumei *et al.* 2004 [48]	rat	OB cells	space shuttle	SPACE	4d & 5d	RT-PCR
Saito *et al.* 2003 [28]	mouse	MC3T3-E1	clinostat	EARTH	3d	RT-PCR
Nakamura *et al.* 2003 [47]	human	OB cells	clinostat	EARTH	12-24-48-96h	RT-PCR
Kumei *et al.* 2003 [46]	rat	OB cells	space flight	SPACE	4d & 5d	qRT-PCR
Kumei *et al.* 2003 [45]	rat	OB cells	space flight	SPACE	4d & 5d	qRT-PCR
Ontiveros and McCabe 2003 [29]	mouse	MC3T3-E1	RWV	EARTH	1d	Total RNA-Real time PCR
Kumei *et al.* 2002 [60]	rat	OB cells	space flight	SPACE	5d +6d	qRT-PCR
Rucci *et al.* 2002 [44]	rat	ROS.SMER# 14	RWV	EARTH	2d	PT-PCR
Landis *et al.* 2000 [43]	chicken calvaria	OB cells	STS-59	SPACE	3d & 5d	Total RNA-cDNA(northern blot)
Sato *et al.* 1999 [31]	rat	MC3T3-E1/ HeLa cells	clinostat	EARTH	2d	NORTHERN BLOT
	-		TR-1A6 (rocket)	SPACE	6min	RT-PCR
Hughes *et al.* 1998 [32]	rat	MC3T3-E1	STS-76	SPACE	29h	RT-PCR
Carmeliet *et al.* 1997 [42]	human	MG-63	FOTON-10	SPACE	9d	RT-PCR ,Northern blot
Kumei *et al.* 1996 [41]	rat	OB cells	STS-65	SPACE	5d	qRT-PCR
Bikle *et al.* 1994 [35]	rat	OB cells	HLS	EARTH	2w	Northern blot
	-	OB cells(tibia)	STS-54	SPACE	6d	Northern blot

(RWV: Rotating Wall Vessel, HARV: High Aspect Rotating Vessel, HLS: Hind-Limb Suspension, ISS: International Space Station, RCCS: Rotary Cell Culture System, HDBR: Head Down Bed Rest, LG-HMF: Large Gradient High Magnetic Field, RPM: Random Positioning Machine, OB: Osteoblast, RT-PCR: Reverse Transcription Polymerase Chain Reaction, HiSeq DNA: High Sequencing DNA, HLU: Hind-Limb Unloading, 2D: two Dimension, 3D: three Dimension, d: Days, h: Hours, w: Weeks, min: Minutes, qRT-PCR: Quantative : RT-PCR, cDNA: Complementary DNA).

4. DISCUSSION

Bone regulation is determined by the synergic action of specific cells that are the osteoblasts and osteoclasts. The coupled functions of these cells, which respond to environmental stimuli, determine bone production and resorption [60, 61]. In conditions of decreased gravity, the change in the environment which the aforementioned cells were normally adept, cause alterations in gene expression and protein production in an effort to accommodate for the new conditions, and results in loss of bone mass during space flights [1, 5, 35]. The mechanism of this phenomenon, that is not yet fully elucidated, involves alterations in the process of genesis, differentiation, proliferation and maturation of osteoblasts and osteoclasts.

Most research has focused on the effect of μG on osteoblasts and not as many studies have been performed that are addressing the osteoclasts. In order to understand the cellular processes that induce osteopenia, when there is exposure to microgravity, experimentation in ground based facilities as well as in space have been conducted. Different devices that employ different principles, have been used to simulate microgravity, like magnetic levitation using high magnetic field, hind limb suspension, rotation around one or two axis and head down bed rest. Unfortunately due to the high cost of launching a space shuttle, experiments

Table 2. Study characteristics for osteoclastic cell lines.

References	Study Design					Genomic Data
	Organism	Cell Type	Type of µG	Location	Duration	
Ethiraj *et al*. 2018 [11]	mouse	RAW264.7	RCCS	EARTH	24h	RT-PCR
Shanmugarajan *et al*. 2017 [12]	mouse	RAW264.7	RWV	EARTH	5d	RT-PCR
Sambandam *et al*. 2016 [13]	mouse	RAW264.7	RCCS	EARTH	24h	RT-PCR
Chatani *et al*. 2016 [14]	medaka fish	OSC cells	ISS	SPACE	2d	HiSeq
Chatani *et al*. 2015 [15]	medaka fish	OSC cells	ISS	SPACE	60d	Whole transcriptome analysis
Yu-Long *et al*. 2015 [16]	mouse	RAW264.7	LG-HMF	EARTH	48h	RT-PCR
Sambandam *et al*. 2014 [19]	mouse	RAW264.7	RCCS	EARTH	24h	RT-PCR
Saxena *et al*. 2011 [17]	mouse	RAW264.7	RCCS	EARTH	24h	RT-PCR
Sambandam *et al*. 2010 [18]	mouse	RAW264.7	RCCS	EARTH	24h	Microarray(Agilent)
Tamma *et al*. 2009 [20]	mouse	(marrow macro-phages) OSTEO	FOTON - M3	SPACE	10d	RT-PCR
	mouse	PITS (mature OSC)	-	SPACE	4d	RT-PCR
Makihira *et al*. 2008 [21]	mouse	RAW264.7	RPM	EARTH	7d	RT-PCR

(RCCS: Rotary Cell Culture System, RWV: Rotating Wall Vessel, ISS: International Space Station, LG-HMF: Large Gradient High Magnetic Field, HARV: High Aspect Rotating Vessel, HLS: Hind-Limb Suspension, RPM: Random Positioning Machine, OB: Osteoblast, RT-PCR: Reverse Transcription Polymerase Chain Reaction, d: days, h: hours, HiSeq: High Sequencing).

Table 3. Functional annotations of genes reported with respect to osteoblastogenesis (OBgenesis), osteoblast (OBS) differentiation maturation and osteoblast mineralization.

Function	OBgenesis	OBS Differentiation-maturation						OBS Mineralization			
Genes / Studies	RunX2	Osterix	ALP	RANKL	OPG	CollaI	OCN	Pthr1r	Omd	OP	ON
Makihira *et al*. 2008 [21]	U (max d3)	U (max 7d)	-	U (d1) D (d3+d7)	U (d7 little)	U (steady)	-	-	-	-	-
Bucaro *et al*. 2007 [25]	(ND)	-	D	-	-	(ND)	(ND)-	-	-	(ND)	-
Bucaro *et al*. 2004 [27]	D	-	D	-	-	D	D	-	-	-	-
Ontiveros and McCabe 2003 [29]	D	-	D	-	-	-	D	-	-	-	-
Hu *et al*. 2017 [33]	D (until d3)	D (max d2)	D (max d2)	-	-	-	-	-	-	-	-
Shuang *et al*. 2012 [37]	D	-	D	-	-	-	D	-	-	-	-
Pardo *et al*. 2005 [51]	D	-	D	-	-	-	-	D	D	-	-
Patel *et al*. 2007 [53]	D	-	D	-	-	-	-	D	D	-	-
Capuli *et al*. 2009 [55]	D	-	-	U	D	-	-	-	-	-	-

(Table 3) contd….

Function	OBgenesis	OBS Differentiation-maturation						OBS Mineralization			
Genes / Studies	RunX2	Osterix	ALP	RANKL	OPG	ColIaI	OCN	Pthr1r	Omd	OP	ON
Bikle *et al*. 1994 [35]	-	-	U (max2d)	-	-	-	D (max5d)	-	-	-	-
Kapitonova *et al*. 2013 [39]	-	-	U	-	-	U (NS)	U	-	-	NS	NS
Carmeliet *et al*. 1997 [42]	-	-	D	-	-	(D)	D min answer to stimulus.	-	-	-	-
Rucci *et al*. 2002 [44]	-	-	U	-	-	-	U	-	-	U	NA
Rucci *et al*. 2007 [52]	NA	NA	NA	U	D	NA	NA	-	-	NA	-
Landis *et al*. 2000 [43]	-	-	-	-	-	D	D	-	-	-	-
Dai *et al*. 2013 [38]	D (+after BMP2 stimulus)	-	-	-	-	-	-	-	-	-	-
Saito *et al*. 2003 [28]	-	-	-	-	-	D	-	-	-	-	-
Chatani *et al*. 2016 [14]	-	U	-	-	-	U	U	-	-	-	-
Hughes *et al*. 2006 [26]	-	-	-	-	-	-	D	-	-	-	-
Hughes *et al*. 1998 [32]	-	-	-	-	-	-	U	-	-	-	-
Kumei *et al*. 2006 [50]	-	-	-	-	-	-	-	-	-	D	U (little)

(d: day, NS: Not Significant, ND: No Difference, NA: Not Affected, max: Maximum, min: Minimum, D: Down, U: Up, Omd: Osteomodulin, OP: Osteopontin, ON: Osteonectin, Pth1r: Parathyroid Hormone Receptor 1, ColIaI: Collagen Ia, ALP: Alkaline Phospahtase, OPG: Osteoprotegerine, RANKL: Receptor Activator of NF-κB Ligant, OCN: Osteocalcin).

Table 4. Expression of cytoskeletal genes.

-	Cytoskeleton												
	SPTBN1	WASF2	WIPF1	Supervillin	Destrin	Tallin	Paxillin	Vimentin	Actin	alpha-tubulin	fibronectin	HSP73	VEGF (+)
Dai *et al*. 2013 [38]	-	-	-	-	-	-	-	-	D	-	-	-	-
Qian *et al*. 2009 [36]	U	U	U	U	-	U	U	-	-	-	-	-	-
Shuang *et al*. 2012 [37]	-	-	-	-	U (max 8d)	-	-	-	-	-	-	-	-
Guignandon *et al*. 2014 [40]	-	-	-	-	-	-	-	-	-	-	-	-	D
Kumei *et al*. 2003 [46]	-	-	-	-	-	-	-	-	-	-	-	D	-
Kumei *et al*. 2006 [50]	-	-	-	-	-	-	-	-	-	D (5d)	-	-	-

(U: Up, D: Down, d: Days, max: Maximum).

Table 5. Expression of apoptosis- and proliferation-related genes.

Function	Apoptosis											Proliferation Apoptosis			
-	Bax(+)/ Bcl2(-)	P53(+)/ p21(-)	ERK ½ (-)	iNOS(+) /GTPCH	Caspase8	Caspase3	XIAp (-)	HSP70 (-)	PAF-R(-)	CD44 (-)	Akt(-)	c-fos	c-jun	c-myc	MAPK
Buccaro *et al.* 2007 [25]	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hughes *et al.* 2006 [26]	D/D	-	-	-	-	-	-	-	-	-	-	-	-	D	-
Rucci *et al.* 2002 [44]	ND	ND	-	-	ND	-	-	-	-	-	-	-	-	-	-
Rucci *et al.* 2007 [52]	-	-	U	-	-	-	-	-	-	-	-	-	-	-	-
Nakamura *et al.* 2003 [47]	U/U	-	-	-	ND	ND	U	-	-	-	-	-	-	-	-
Blaber *et al.* 2013 [56]	-	D/U	-	-	-	-	-	-	-	-	ND	D	-	-	D
Kumei *et al.* 2003 [45, 46]	-	-	-	U/U	-	-	-	D	U	-	-	-	-	-	-
Sato *et al.* 1999 [31]	-	-	-	-	-	-	-	-	-	-	-	D	ND	-	ND
Chatani *et al.* 2016 [14]	-	-	-	-	-	-	-	-	-	-	-	U	U	-	-
Kumei *et al.* 2007 [54]	-	-	U	-	-	-	-	-	-	-	-	-	-	-	U
Kumei *et al.* 2006 [50]	-	-	U	-	-	-	-	-	U	U	-	-	-	-	U
Buccaro *et al.* 2004 [27]	D	-	-	-	-	-	-	-	-	-	D	-	-	-	-

(ND: No Difference, U: Up, D: Down).

Table 6. Expression of cytokine- and morphogenesis-related genes.

	Cytokines						Morphogenic Proteins	
-	IL-6	Lcn2	MMP10	Cox 2	Cox 1	PGE-2	BMP4	BMP2
Blaber *et al.* 2013 [56]	-	-	U	-	-	-	-	-
Hughes *et al.* 2006 [26]	-	-	-	D	-	U	-	-
Kumei *et al.* 1996 [41]	U	-	-	-	-	U	-	-
Rucci *et al.* 2002 [44]	U	-	-	-	-	-	U	ND
Capulli *et al.* 2009 [55]	U (max 5d)	U (max 5d)	-	-	-	-	-	-

(Table 6) contd….

	Cytokines						Morphogenic Proteins	
-	IL-6	Lcn2	MMP10	Cox 2	Cox 1	PGE-2	BMP4	BMP2
Rucci *et al*. 2015 [57]	-	U (12d) In humans	-	-	-	-	-	-
Rucci *et al*. 2015 [57]	-	U	-	-	-	-	-	-
Patel *et al*. 2007 [53]	-	-	-	-	-	-	D	-
Saito *et al*. 2003 [28]	-	-	-	-	-	-	-	-
Kumei *et al*. 2007 [54]	-	-	-	-	-	U	-	-
Kumei *et al*. 2003 [46]	-	-	-	-	-	-	-	-
Kapitonova *et al*. 2013 [39]	D	-	-	-	-	-	-	-
Hughes *et al*. 1998 [32]	-	-	D	D	ND	-	-	-
Pardo *et al*. 2005 [51]	-	-	-	-	-	-	D	-

(d: Days, ND: No Difference, U: Up, D: Down, IL: Interleukin, BMP: Bone Morphogenetic Protein, Cox: Cyclooxigenase, Lcn2: Lipocalin 2).

conducted in real μG are limited and sparse. The duration of exposure to μG among studies varies from 1 day to 8 weeks and in most cases cell cultures are used, with less studies being conducted in living organisms. Other concomitant factors that can affect as well the response to μG, are the hypergravity induced at launch and cosmic radiation exposure.

4.1. Microgravity and Osteoblasts

Multipotential mesenchymal cells differentiate into osteoblastic lineage cells under the effect of transcription factors runx2 and ostx. Condensed progenitor osteochondrocytes divert to pre-osteoblasts that express high level of runx2 and under the effect of osx mature to functional osteoblastic cells that produce specific marker proteins ALP, CollI, OCN of the osteoblastic cell line [62]. All ground based and space studies agree that there is a downregulation of runx2 when pre-osteoblastic cells are exposed to microgravity conditions. Runx2 is expressed by pre-osteoblastic cells exposed to μG, yet there is a delay of onset in the production as shown by Makihira *et al.* [21], suggesting that cell differentiation is a slower but ongoing process. In the process of maturation, ostx's input is important for creating functional osteoblastic cells. Both in real and simulated conditions of μG, expression of the later was detected with a time depended rhythm, but overall levels had less statistical importance. Thus, the results indicate that differentiation is inhibited initially but recovers in the long run. Osteoblast-osteoclast interaction is mediated by the production of soluble and intermembrane RANKL by the osteoblasts. RANKL fuses with RANK receptor on the surface of the osteoclasts and therefore activating them. The action of RANKL is only nullified by connecting with OPG, a protein that is also produced by the osteoblasts [60]. In microgravity RANKL levels show upregulation early when exposed (day 1), while OPG follows a slow pattern of progressive production lower than in normal gravity. Even though the levels of RANKL and OPG are not altered in significant degree, the overall ratio of RANKL/OPG reaches significance. The early upregulation of RANKL and the overall downregulation of OPG, can be interpreted as a cellular response to the sudden change in gravitational conditions, diverting homeostasis towards bone resorption. Maturated osteoblasts are capable of producing large quantities of ALP, ColI and OCN so as to form bone matrix which in turn will be mineralized. This process is inhibited in μG, as studies indicate downregulation of ALP and ColI, as well as OCN, indicating an inhibition in the maturation of osteoblasts. Interestingly there are also reports of OCN upregulation as early as the 2nd day of exposure [44]. This can be explained if we consider that high levels of OCN create an anionic environment, which is necessary for osteoclastic activity [39]. Furthermore, the endocrine role of OCN is related to glucose regulation, as it increases insulin release and targets cell sensitivity towards insulin [63]. Even though it appears that μG does not affect the production of insulin growth factor (IGF), cells develop insulin resistance. The increased OCN as well as insulin growth factor receptor(IGF-R) levels can be explained as an effort to counteract the former phenomenon [35].

Osteoblasts are mechanosensitive cells that respond to gravitational alterations. Extracellular signals are perceived by transmembrane molecules and converted into intracellular signals, by a complex mechanism, causing changes in gene expression in the nucleus. Proteins of the cytoskeleton as well as cytokines and growth factors contribute significantly to this intricate metabolic pathway, *via* signal transduction. Growth factors and cytokines attach to their intermembrane receptors thus activating intracellular responses [26, 64]. Proteins of the cytoskeleton talin, paxillin, supervillin, WASF2, WIPF1 were upregulated when exposed to altered gravitational conditions 0g *versus* 2g, *via* large gradient diamagnetic field for 24 hours [36]. WASF2, which is a downstream effector of cell division cycle 42(Cdc42) that is implicated in actin polymerization and cytoskeletal organization as well as WIPF1that encodes a protein responsible for actin polymerization [65], were more sensitive in alterations of gravity than the magnetic field [36]. Kumei *et al.*, 2006 found no alteration in actin or β1-integrin expression levels in rat osteoblasts cultured for 4-5days in space. In the same

Table 7. Expression of cytokine- and morphogenesis-related genes.

Function	Study Gene (Protein)	Ethiraj 2018 [11]	Sanmugarajan 2017 [12]	Sambandam 2016 [13]	Chatani 2016 [14]	Chatani 2015 [15]	Yu-Long 2015 [16]	Saxena 2011 [17]	Sambandam 2010 [18]	Tamma 2009 [20]	Makihira 2008 [21]	Sambandam 2014 [19]
Osteoclast Markers	RANK	-	-	-	-	-	-	U (with RANKL)	-	-	-	-
	TRAP	-	U	-	U	-	Suppr	-	-	U	-	-
	MMP-9	-	-	-	U	-	U	-	U	U	-	-
	Cathepsin-K	-	-	-	U	-	Suppr	U	U	U	-	-
	CaIR	-	-	-	-	-	-	U	-	U	-	-
Fusion- Differentiation-Proliferation	Syncytin-A	U	-	-	-	-	-	-	-	-	-	-
	Syncytin-B	ND	-	-	-	-	-	-	-	-	-	-
	Dcstamp	-	U	U	-	-	-	-	-	-	U	-
	Ocstamp	-	U	U	-	-	-	-	-	-	-	-
	CCN2/CTGF	-	U	-	-	-	-	-	-	-	-	-
	TRAF6	-	-	U	-	-	-	-	-	-	-	-
	TRAIL (TNF re-lated)	-	-	U	-	-	-	-	-	-	-	-
Transcription Factors	Runx2	-	-	-	-	-	Suppr	-	-	-	-	-
	NFATc1	-	-	-	-	-	U	U(without RANKL)	-	-	-	-
	Pcreb	-	-	-	-	-	-	-	U	-	-	-
Adhesion	Podoplanin	-	-	-	-	-	-	-	U	-	-	-
	B-integrin	-	-	-	-	-	-	-	U	U	U (with-out RANKL) Down (with RANKL)	-
Signal Transduction	S100A8	-	-	-	-	-	-	-	U	-	-	-
Nucleus & mitochondrial Signaling	c-fos	-	-	-	U	-	-	-	-	-	-	-
	Jun-b-like	-	-	-	U	-	-	-	-	-	-	-
	Pai-1	-	-	-	U	-	-	-	-	-	-	-
	ddit4	-	-	-	U	U	-	-	-	-	-	-
	Fkbp5	-	-	-	-	U	-	-	-	-	-	-
	Tsc22d3	-	-	-	U	-	-	-	-	-	-	-
Autophagy	LC3	-	-	-	-	-	-	-	-	-	-	U
	Atg5	-	-	-	-	-	-	-	-	-	-	U

(Suppr: Suppressed, ND: No Difference, U: Up).

study osteopontin levels decreased while CD44 increased. Integrins and CD44 are transmembrane adhesion molecules that are interconnected with actin filament, and the interaction between osteopontin (extracellular) and CD44 is mediated by integrins [66]. Disruption of the actin cytoskeleton alters cellular response to morfogenetic proteins. *Dai et al.* 2013 demonstrated that BMP2 proliferative effect is inhibited when actin microfilament is disrupted [38], which supports the mechanosensation role of the cytoskeleton. Changes in gravity perceived by proteins of the cytoskeleton alter cellular response to the effect of morphogenic proteins, such as BMP2, thus inhibiting osteoblast differentiation [38].

Interactions between osteoblasts and osteoclast mediated by soluble molecules such as cytokines contribute to bone homeostasis and regulation of bone production and resorption. IL-6 is a pleiotropic cytokine that is implicated in the regulation of bone turnover, by regulating osteoblast and osteoclast differentiation and function [67]. It is produced by T lymphocytes, monocytes, osteoblast/stromal cells, fibroblasts, synovial cells and cancer cells and consist of a family of 10 factors, IL6 , IL11, IL27, IL31, Leukemia Inhibitory Factor (LIF), Oncostatin M (OSM), Ciliary Neurotrophic Factor (CNTF), Cardiotrophin-1 (CT-1), Cardiorophin Like Cytokine (CLC) and Neuropoietin (NP) [68-72]. IL-6 binds to a specific subunit IL-6Ra, which is either membrane or soluble (sIL-6Ra) and that complex interacts with two gp-130 molecules and forms a hexameric complex [73, 74]. In bone, soluble IL-6R is required for IL-6 effects on osteoclast development and osteoblast function but little is known of its origin. IL-6 might be produced by other cell types, like liver cells or by osteoblasts [75, 76]. All IL-6 cytokines use the transducing receptor β-subunit gp 130, which further activate Janus Protein-Tyrosin Kinases (JAKs). In turn this allows the activation of the Signal Transducer and Activator of Transcription (STATs) or of the MAPKs [77]. Activation of STAT3 is necessary for osteoblast differentiation and bone formation induced by IL-6, but it can also promote the expression of cell cycle inhibitor p21, which has shown to confer resistance to apoptosis in osteoblastic cells [78, 79]. On the other hand other *in vitro* reports have shown that IL-6 complex has inhibitory effects on bone formation *via* extracellular signal regulated kinase 1/2(ERK1/2) and PKCδ kinases (MAPKs pathway). Furthermore the transcription factor STAT5a/b and p53 act in synergy to enhance bax/bcl ration and sensitize osteoblastic cells to apoptosis [80, 81]. These dual effects of IL-6 could depend on the differentiation stage of osteoblasts, but considering that osteocytes, the final form of osteoblasts, are characterized by decreased production of osteoblastic marker proteins and high apoptotic levels, it is possible that IL-6 complex contributes throughout the osteoblastic cell life cycle [82, 83]. IL-6 role in osteoclast differentiation is performed by increasing interactions between osteoblasts and osteoclasts. The effect of IL-6 complex induces production of RANKL, PGE2, IL-1 by osteoblasts inducing osteoclast differentiation. Recent data suggest that STAT3 has a key role in RANKL production by the osteoblasts, under the effect of IL-6 [84-86]. In contrast, other studies report that IL-6 has inhibitory effects on osteoclast formation by diverting cells into the macrophage lineage. That action is mediated by inhibiting RANKL pathways through activation of NF-κB and MAPKs [86, 87]. It appears that the activity of the receptor complex of IL-6 is the result of the antagonistic action of STAT *versus* MAPK pathways, where STAT exerts apoptotic and anti -proliferative effects on osteoblast and osteoclasts and MAPK has mitogenic and anti-apoptotic [68, 70, 86]. In studies of simulated or real μG, IL-6 was found upregulated in three and downregulated in one. All experiments were *in vitro* cell cultures of the osteoblast lineage and it was interesting that down regulation of IL-6 was noted in real μG in space, while upregulation in simulated conditions. The complex actions of this cytokine as well as the fact that it can be produced by various cell types and contribute to a great number of biologic processes, precludes the necessity of *in vivo* study designs, in order to elucidate the whole spectrum of IL-6 complex effects on bone metabolism in microgravity environment.

Cellular response to stress induced by μG, leads to changes in the expression of apoptotic and anti-apoptotic genes. Concerning cell survival, studies concluded that microgravity does not directly induce apoptosis. In *in vitro* and *in vivo* experiments under real or simulated μG, expression of apoptosis- related genes and anti-apoptotic genes were concomitantly downregulated or showed no difference in expression with statistical importance [25, 27, 44, 46, 56]. Interestingly osteoblastic cells that were exposed to μG, became sensitized to apoptotic agents like staurosporin [27, 56] but no sensitivity was detected to the apoptotic agent sodium nitroprusside [25]. Bax mitochondrial apoptotic gene and its counterpart Bcl were increased concomitantly in one study [47], while under real microgravity on board the space shuttle, downregulation was noted which was normalized in 1g [26]. The aforementioned genes are expressed and regulate mitochondrial function. Bax releases cytochrome c and induces apoptosis through p53 tumor suppressor gene, while Bcl-2 blocks the apoptosis mechanism by stabilizing mitochondrial membrane, thus inhibiting the release of cytochrome c [88, 89]. Several anabolic signals are gravity dependent and can be downregulated in microgravity. The MAPK pathway is the central controlling point for signal transduction from the extracellular environment to the nucleus of osteoblasts [90]. The extracellular signal-regulated kinase (ERK)-mitogen-activated protein kinase (MAPK) pathway provides a major link between the cell surface and nucleus to control proliferation and differentiation [61]. Mechanical stress regulates Runx2 activation and favors osteoblast differentiation through the activation of MAPK signal transduction pathways and Ras/Raf-dependent ERK1/2 activation, but is independent of p38 MAPK signaling [91]. Apoptosis of osteoblast mouse cells did not increase after 24 hours of clinorotation but it was increased after the introduction of MEK inhibitor PD98059, suggesting a protective role of ERK1/2 [52]. MAPK activity is induced by gravity and causes upregulation of c-fos within 30 minutes of stress. This activation is inhibited by MEK kinase inhibitor but not a p38 inhibitor. Blocking the MAPK signaling pathway and MEK1 also promotes differentiation of MC3T3-E1 pre-osteoblastic cells to more mature forms, that produce ALP and collagen I, suggesting that this pathway may influence the differentiation process in osteoblasts [92]. ERK ½ phosphorylation causes translocation of ERK to the nucleus and NF-κB mediated c-fos gene activation [90]. Members of the Fos, Jun and ATF family of proteins form the complex of transcrip-

tion factors AP-1 that are implicated in bone regulation. Various members of the AP-1 complex are differentially expressed during osteoblast maturation, with high levels of c-fos and a-jun being expressed *in vitro* during osteoblast maturation. Chimeric mice obtained from c-Fos overexpressing embryonic stem cells develop chondrogenic tumors implying a function of c-Fos in chondrogenesis *in vivo* [93]. Furthermore, C-fos knock out mice showed an osteopetrotic phenotype with a shift of osteoclasts to bone marrow macrophages [93, 94]. C-fos expression is more marked during the proliferation phase of osteoblasts, and diminishes during mineralization. Over- or under- expression of this transcription factor is consistent with abnormalities in bone development [95]. In space conducted *in vivo* studies c-fos was upregulated early (2 days) [14], as well as ERK ½ in real or simulated μG experiments, while p38 and JNK expression did not significantly change [52, 54]. On the other hand, EGF(epidermal growth factor) -induced c-fos expression was restrained about 30% compared to ground controls, after short-term simulated microgravity and rocket flight, when no difference in MAPK phosphorylation was noted [31]. The potential role of the intricate metabolic pathway of MAPKs, in bone regulation under stressful conditions such as microgravity is far from being elucidated. Yet it is clear that it plays an important part in moderating osteoblast cell cycle, differentiation, proliferation and maturation processes.

4.2. Microgravity and Osteoclasts

In contrast to the plethora of experimental data concerning the effect of microgravity in cells of the osteoblastic lineage, osteoclast data is limited. Few short-term studies (8 in total) of simulated μG and two performed in real μG (Table **2**) have provided insight to the changes in osteoclast expression in weightlessness conditions. Early exposure to μG induces expression of nucleus genes c-jun, c-fos, jun-B-like as well as proteins of the MAPK pathway (ERK, p38, JNK) which under the effect of RAKL result in the formation of gigantic multinucleated osteoclast cells [14, 17, 18]. These cells express high level of osteoclast marker proteins TRAP, MMP9, cathepsin K and Calcium receptor. Fusion process in premature osteoclasts is a complex process that still remains unclear. It has been suggested that osteoclasts chose their partners selectively according to their maturation stage and organization of fusion factors [96]. Mensah *et al.* [97] supported that fusion is determined by the presence or absence of DCSTAMP on extracellular membrane, while in another report syncytin-1 formed a concentrated pattern in the area facing the fusion cell [98]. It is possible that osteoclasts do not randomly choose their partner but this process is based on selectivity among a heterogeneous population based on complementarity and maturation stage [98, 99]. In different stages of osteoclast nuclearity, different fusion proteins are the prime orchestrators. Syncytin-1 promotes fusion of multi-nucleated cells and reduces the number of fusions between mono-nucleated cells of pre-osteoclasts [99]. In microgravity studies the levels of adhesion proteins participating in cell fusion DCSTAMP and OCSTAMP were increased. Microgravity was the main contributor rather than radiation and under the effect of RANKL cell fusion was noted as early as 24 hours after exposure. This resulted in the formation of mature osteoclasts presenting larger numbers of nucleuses [12]. B-integrin's action was also RANKL dependent and was enhanced in microgravity [21]. Another contributor to the process of osteoclast fusion is syncytin-A, which was also upregulated in μG, independent of RANKL (Table **7**). When syncytin-A was blocked, cell population expressing TRAP protein was diminished and mechanism of autophagy was negatively influenced even at the presence of RANKL [11]. Interestingly OCSTAMP and DCSTAMP were found upregulated through another pathway independent of RANKL. Sambandam *et al.* [13], noted that through the inflammatory pathway of TNF family the upregulation of TRAIL is able to increase OS and DC STAMP levels independent of RANKL. This family of inflammatory proteins may also participate in autophagy mechanisms. Autophagy is a cellular self- consumption process that is involved in cell survival, nutrient supplementation under starvation, antigen presentation and defense against harmful agents [100]. In osteoclasts autophagy proteins (Atgs/LC3) regulate the formation of autophagosomes. In addition, the same proteins regulate the secretory lysosomes that are directed towards the ruffled area of the osteoclasts where osteoid degradation takes place [100]. Disruption of autophagy was shown to delocalize cathepsin K and reduce bone resorption [101]. Tnfs10 mRNA levels were markedly elevated in RAW 264.7 cells that were cultured for 24 hours in clinorotation. Recent studies have shown implication of TNF inflammatory cytokines in the induction of autophagy mechanisms [102] In the same study Atg5 and LC3 autophagy markers were upregulated without the effect of RANKL. The introduction of 3-MA, an autophagy inhibitor, reduced cathepsin K levels, Atg5 and LC3, therefore debilitating osteoclastic activity, without affecting their viability [19].

CONCLUSION

Our understanding of microgravity's effect on bone metabolism has increased, with the accumulation of data from many experiments, in real or simulated conditions. Osteoblasts appear as the main orchestrators of bone metabolism in microgravity and the intricate metabolic pathway of MAPK seems to play protagonistic role in microgravity induced alterations in the osteoblastic lineage cells. Osteoclasts become sensitized in microgravity and their activity is augmented by RANKL produced by the osteoblasts as well as through inflammatory cytokines. Still we are far from fully elucidating the mechanism that causes space osteopenia. We are now at a turning point for humanity's future regarding space exploration. Advances in robotics and biotechnology offer possibilities of performing studies in space that could improve life on Earth. Unraveling the pathophysiologic mechanisms that cause μG-induced changes in the skeletal, immune, cardiovascular and other systems of human organism may lead to the development of more efficient therapies for a variety of diseases. Furthermore, the dream of reaching to distant parts of our galaxy can become a possibility, only after the great conundrum of space-induced osteopenia is solved.

LIST OF ABBREVIATIONS

ALP	=	Alkaline Phosphatase
HARV	=	High Aspect Rotating Vessel
HDBR	=	Head Down Bed Rest

HiSeq DNA = High Sequencing DNA
HLS = Hind-Limb Suspension
HLU = Hind-Limb Unloading
IL = Interleukin
ISS = International Space Station
LG-HMF = Large Gradient-High Magnetic Field
NGS = Next Generation Sequencing
OB = Osteoblast
OCN = Osteocalcin
OPG = Osteoprotegerin (TNF receptor superfamily member 11b)
RANKL = TNF superfamily member 11
RCCS = Rotary Cell Culture System
RPM = Random Positioning Machine
RT-PCR = Reverse Transcription Polymerase Chain Reaction
RWV = Rotating Wall Vessel
μ G = Microgravity

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