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The role of autophagy during osteoclastogenesis under microgravity conditions

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Review

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Abstract

Space represents a rather hostile environment for the human body, with the bone loss being one of the most important consequences. Autophagy is a complex cellular process contributing to several cellular processes including recycling, nutrition, apoptosis and response to stressful environments. Recent reports have indicated that autophagy is a process that increases under microgravity conditions. In particular, this was shown to be true in skeletal cells such as the osteoclasts. Suppression of autophagy results in downregulation of osteoclastogenesis, making autophagy a quite tempting therapeutic target for preventing bone loss during space flights. The present work attempts to review the literature on the topic of autophagy role in osteoclastogenesis under microgravity conditions.

Introduction

In the thought of space travelling the first thing that comes to mind is the *USS Enterprise*, travelling in with warp speed, equipped with artificial gravitational forces and terrestrial luxuries. Yet, all these still remain in the sphere of science fiction as for example the problem of gravity in a space flight. Further on, another problem that still remains to be elucidated is the effect of lack of gravity on human physiology both on the cellular and systemic level. For example, during a space travel to Mars, which will take approximately 1 year, the cosmonauts will suffer a bone and muscle loss of 19%. Besides the musculoskeletal effects, other effects *n* immune system, cardiovascular also remain largely unknown.

Microgravity experienced by astronauts during spaceflight causes significant physiological alterations in the human body. Bone loss is one of the most important of these problems. The role of gravity in the developing musculoskeletal system is undisputable, yet there is still much to be elucidated. The exact mechanisms are not yet clear that is, how exactly gravitational forces and the gravitational field, signals to cells in order for them to retain their shape, size and function. Since the first space flight, the vision was to succeed a long-term space flight to colonialize other planets, at least in our Solar System. Yet, the first experience on that matter showed that gravitational forces and subsequently their absence lead to significant complications concerning human well-being in space. Several problems emerged and some of them of grave nature, such as osteoporosis and muscle atrophy. This issue has been an area of intensive research in recent years. The more technology develops allowing and facilitating a long-distance space trip the more research on the role of gravity on living organisms intensifies.

Microgravity and bone physiology

Microgravity simulation models

The term '*microgravity*' is defined as the condition in which the sum of all forces producing accelerations does not exceed 10^{-6} $\boldsymbol{g}$ although it is usually mistaken as a synonym to the absence of gravity. Microgravity is actually achieved about 300–400 km over Earth, where accelerations on Moon and Mars have been referred to be 0.17 $\boldsymbol{g}$ and 0.38 $\boldsymbol{g}$, respectively (Herranz *et al.*, 2013).

Research under microgravity conditions is necessary in order to discover the effect of microgravity on biological processes. However, research under such circumstances is constrained by the high cost of space flights. Microgravity simulators on Earth are valuable tools which help scientists to reproduce conditions that resemble microgravity for their experiments (Herranz *et al.*, 2013).

Long duration bed rest is a human microgravity analog used for the disclosure of the impact of microgravity on the human body. Especially, 6° head down tilt bed rest has been proposed by Russian investigators as the most suitable analog for microgravity simulation to study the

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Table 1. Influence of microgravity on bone cells

Bone marrow stem cells	Maintenance of undifferentiated state of cells
	Induction of proliferation
	Differentiation into adipocytes instead of osteoblasts
Osteoblasts	Reduced proliferation
	Impaired differentiation
	Impaired functionality
Osteocytes	Impaired responsiveness to mechanical stimuli
Osteoclasts	Induced differentiation
	Increased absorption activity

impact of microgravity on bone metabolism and muscles, as well as the development of countermeasures used to eliminate the adverse effects (Hargens and Vico, 2016).

The rodent hindlimb unloading model by tail suspension was developed in the 1980s in order to study the effects of space flight on human organ systems, the underlying mechanisms and the countermeasures developed to deal with the detrimental effects on human health (Globus and Morey-Holton, 2016).

The National Aeronautics and Space Administration (NASA) has developed a RWV (Rotating Wall Vessel) in order to study the effects of reduced mechanical forces on cell cultures, as a cellular analog to microgravity. The rotating wall vessel is a suspension culture vessel with a diffusion membrane for gas exchange without bubbles, where the cells are placed on micro-carrier beads and the culture medium rotates in a horizontal axis, which results in reduced shear stress and turbulence. The constant rotation of the culture results in a constant change of gravitational vector, which resembles free fall and some aspects of microgravity (Hammond and Hammond, 2001).

The effect of microgravity on bone

Exposure to real and simulated microgravity results in alterations in bone metabolism (Table 1). A significant problem related to exposure to microgravity is bone loss. Members of space missions in RSS (Russian Space Station) and ISS (International Space Station) have experienced 1% reduction in lumbar spine bone mineral density and 1–1.6% reduction in hip bone mineral density during a month's time, where an equal percent of bone loss is experienced by menopause women in a year (Vico *et al.*, 2000). Significant aspects of gravity influence cellular functions. For example, cells rearrange their cytoskeleton depending on the forces exerted on them and the cytoskeleton is less prominent under microgravity conditions (Arfat *et al.*, 2014). Reduced mechanical forces under microgravity result in increased bone resorption which cannot be compensated by bone formation (Hughes-Fulford, 2003).

There are four main types of bone cells: mesenchymal stem cells, osteoblasts, osteocytes and osteoclasts, which are influenced by microgravity. Microgravity results in maintenance of an undifferentiated state of mesenchymal stem cells induce their proliferation and lead to differentiation into adipocytes instead of osteoblasts (Yuge *et al.*, 2011). ERK kinases play a significant role in the signalling pathway which controls the differentiation of mesenchymal stem cells to adipocytes (Feve, 2005). Modelled microgravity-induced alterations in the cytoskeleton of mesenchymal stem cells, such as thinner F-actin fibres and change of the shape of the cells (Buravkova *et al.*, 2008). Some researchers support that alterations of the cytoskeleton contribute to restrictions in the differentiation of mesenchymal stem cells to osteoblasts (Zayzafoon *et al.*, 2004).

Real and modeled microgravity reduces osteoblastic differentiation, proliferation and activity, as well as their responses to environmental stimuli (Arfat *et al.*, 2014). MC3T3 cell line cultured under modeled microgravity developed less complex cytoskeleton, which is related to the nucleus activity. Besides this, microgravity disturbs microfibres of osteoblasts, which results in less bone formation (Arfat *et al.*, 2014). Carmeliet *et al.* (1997) have proved that alkaline phosphatase expression and activity is significantly reduced under microgravity conditions (Carmeliet *et al.*, 1997).

Osteocytes act as mechanical sensors through their dendritic axons (Bonewald and Johnson, 2008). The sensitivity of osteocytes to mechanical stimuli is reduced under modelled microgravity conditions and F-actin fibres are deranged (Di *et al.*, 2011). Apoptosis of osteocytes is essential in order for bone remodelling to begin (Kennedy *et al.*, 2012). Besides, osteocytes produce sclerostin, which is an inhibitor of Wnt-β catenin signalling pathway, which is crucial in osteoblastogenesis (Lin *et al.*, 2009).

Osteoclast differentiation is induced under microgravity conditions and an increased amount of collagen telopeptides has been found in osteoclast cultures, indicating increased osteoclast activity (Tamma *et al.*, 2009). Sambandam *et al.* (2010) have proved a significant increase in expression of genes involved in osteoclast differentiation using microarray analysis (Sambandam *et al.*, 2010). Nabavi *et al.* (2011) observed an increase in resorption pits in bones under microgravity conditions and a significant increase in osteoclast resorption activity (Nabavi *et al.*, 2011). Modelled microgravity-induced the production of radical oxygen species in preosteoclast cell line RAW264.7, which have a crucial role in bone resorption and osteoclastogenesis induced by RANKL (Lee *et al.*, 2005).

Microgravity and autophagy

Autophagy

Autophagy is a recycling cellular process of eukaryotic cells, which cells use to decompose damaged organelles and other macromolecules into amino acids, sugars, nucleonic acids and then use the 'raw material' to compose new macromolecules or produce the energy they need (Mizushima, 2007). All cells show a basic level of autophagy, which serves as a quality control mechanism for cellular components. The level of autophagy increases under stress conditions, such as fasting periods, oxidative stress, hypoxia or infection, in order to protect the cell from potential damage (Kroemer *et al.*, 2010). It has recently been proved that autophagy is related to cellular life expectancy. Ageing reduces the level of autophagy in cells, whereas inducing autophagy pharmacologically or genetically results in longevity (Carmona-Gutierrez *et al.*, 2016). Defective autophagy has been related to diseases, such as infections, cancer and neurodegenerative diseases (Mizushima *et al.*, 2008).

Impact of autophagy on bone cells

Autophagy disorders have been recently related to bone cell dysfunction (Table 2). Induction of autophagy contributes to the

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Table 2. Impact of autophagy on bone cells

Bone marrow stem cells	Survival through oxidative stress
	Preservation of germination capacity
Osteoblasts	Final stages of differentiation
	Bone mineralization
Osteocytes	High level of basic autophagy (due to food and oxygen deprivation)
Osteoclasts	Differentiation
	Function (bone resorption)

survival of mesenchymal stem cells under oxidative stress circumstances (Song *et al.*, 2014). Autophagy is involved in the preservation of stemness, which is the ability of mesenchymal stem cells to renew themselves and differentiate into different cell types, as it has been observed in human cells from the umbilical cord (Hou *et al.*, 2013). Nuschke *et al.* (2014) have observed aggregated autophagosomes in early stages of differentiation of mesenchymal stem cells, which they consume as energy fuel in order to differentiate into osteoblasts (Nuschke *et al.*, 2014).

Experiments with osteoblast cell lines have proved that autophagy increased the differentiation of osteoblasts and bone mineralization, which is the main activity of osteoblasts. Liu *et al.* have observed that autophagy is important in terminal stages of osteoblastogenesis, without being implicated in proliferation or early stages of osteoblast differentiation (Liu *et al.*, 2013). *Nollet et al. (2014)* have observed autophagosomes containing hydroxyapatite in osteoblasts. Osteoblasts use autophagy as a mechanism for exocytosis of hydroxyapatite, indicating the important role autophagy plays in bone mineralization (Nollet *et al.*, 2014).

Osteocytes, because of their long life and their location being embedded in the mineralized bone matrix, face a constant state of stress, which results in a high level of basic autophagy. Zahm *et al.* (2011) have observed a punctuate distribution of LC3, which is an autophagy marker, indicating a high level of basic autophagy in murine and human cortical bone osteocytes. They also observed induction of autophagy under starvation and hypoxia, conditions that are common in the environment of osteocytes (Zahm *et al.*, 2011). Yang *et al.* (2014) proved that ovariectomy in murines resulted in increased oxidative stress and level of autophagy in osteocytes, as a result of estrogen deprivation (Yang *et al.*, 2014). Low doses of corticosteroids stimulate autophagy in osteocytes, as a mechanism to survive the stress caused by steroids (Jia *et al.*, 2011).

Autophagy and proteins taking part in the signalling pathway of autophagy have a significant role in differentiation and function of osteoclasts (Pierrefite-Carle *et al.*, 2015). Wang *et al.* (2011) proved that upregulation of Beclin-1 and autophagy play an important role in osteoclastogenesis induced by MCP-1. Knocking down Atg7 suppressed the expression of TRAP and cathepsin K, which are osteoclast markers, indicating that autophagy is involved in osteoclastogenesis (Wang *et al.*, 2011). Chung *et al.* (2014) showed that Beclin-1, which is an autophagy marker, induces reactive oxygen species formation and NFATc1 expression, which is an important transcription factor in the signalling pathway of osteoclastogenesis (Chung *et al.*, 2014). TRAF3, which suppresses osteoclastogenesis induced by RANKL, is degraded by autophagy in pre-osteoclasts (Xiu *et al.*, 2014).

In addition, proteins taking part in the signalling pathway of autophagy, such as Atg5, Atg7, Atg4B, LC3, are necessary for the formation of the ruffled border of mature osteoclasts and their resorption activity. Atg5 is required for the transport of LC3II to the ruffled border, where LC3II promotes the fusion of lysosomes, which is necessary for the resorption activity of osteoclasts (DeSelm *et al.*, 2011). Chung *et al.* proved that osteoclast activity is related to the conversion of LC3I to LC3II, but not to the level of autophagy. Suppression of LC3 did not interfere with the formation of multinucleated cells expressing TRAP, which is an osteoclast marker. Instead, suppression of LC3 repressed the F-actin ring formation and exocytosis of cathepsin K, resulting in a reduction of osteoclast bone resorption activity (Chung *et al.*, 2012).

Autophagy and microgravity

It has been noted since 1980s that microgravity modifies organ function of organisms subjected to microgravity. Research on the underlying mechanisms has started since then (Rokhlenko and Mul'diiarov, 1981). Rokhlenko and Mul'diiarov (1981) first described the changes in myocardial cells of rats exposed to microgravity and noted the presence of more autophagosomes in animals exposed to microgravity than in those on earth (Rokhlenko and Mul'diiarov, 1981). Riley *et al.* (1987) supported that autophagy does not play an important role in microgravity because they found no significant increase in lysosomes in skeletal muscle cells of rats exposed to microgravity (Riley *et al.*, 1987). Thirty years later, autophagy is reevaluated as a mechanism responsible for adaptation of cells to exposure to microgravity.

Risks posed by microgravity to the cardiovascular system have been a challenging subject for researchers to study. Wang *et al.* (2013) observed that autophagy is upregulated in endothelial cells of the human umbilical vein under exposure to modelled microgravity for 48 h, as indicated by increased autophagosomes and expression of beclin-1 and LC3, which are autophagy markers (Wang *et al.*, 2013). Zhong *et al.* (2016) included autophagy in a review describing pathophysiological mechanisms responsible for cardiac atrophy under microgravity conditions (Zhong *et al.*, 2016).

Influence of microgravity on the musculoskeletal system has been extensively studied in order to develop compensatory measures. Sandona *et al.* (2012) investigated skeletal mouse muscles which were exposed to microgravity during a 90-days space mission. They observed that muscle atrophy was more obvious in muscles that support weight-bearing bones than muscles which are responsible for other movements, but there was not a statistically significant difference in expression of genes (LC3b and cathepsin L) which are considered autophagy markers (Sandona *et al.*, 2012). Ryu *et al.* (2014) observed an increased number of autophagosomes and expression of LC3II using Western blot analysis in myoblast cell cultures exposed to modeled microgravity for 72 h. They reached the conclusion that autophagy is induced under microgravity conditions and is probably involved in the pathogenesis of muscle atrophy under such circumstances (Ryu *et al.*, 2014).

Yoo *et al.* (2016) proved that levels of LC3II are time-dependently increased in preosteoblast cells MC3T3-E1 during their exposure to modeled microgravity for 72 h, which was attributed to increased phosphorylation of proteins ERK/Akt/mTOR. They also studied the effect of melatonin on the expression of autophagy markers and reached the conclusion that melatonin counteracts the effect of microgravity on preosteoblast autophagy markers' expression (Yoo *et al.*, 2016).

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Table 3. Microgravity and autophagy

References	Cell culture	Method	Change
Rokhlenko *et al.* (1981)	Rat myocardium	Microscopy	No impact
Riley *et al.* (1987)	Rat skeletal muscles	Microscopy	Scarce lysosomes
Sandona *et al.* (2012)	Mouse skeletal muscles	LC3b, cathepsin L	Not changed
Wang *et al.* (2013)	Human endothelial cells of umbilical vein	Autophagosomes, Beclin-1, LC3-II	Increased
Ryu *et al.* (2014)	Myoblasts	LC3-II	Increased
Sambandam *et al.* (2014)	Preosteoclasts RAW264.7	Atg5, LC3-II	Increased
Ryu *et al.* (2014)	Human embryonic renal cells HEK293	LC3-II P62	Increased Decreased
Ferranti *et al.* (2014)	Seminoma Tcam-2	LC3-II	Temporarily increased
Indo *et al.* (2015)	Neuroblastoma	BECN1	Not changed
Yoo *et al.* (2016)	Preosteoblasts MC3T3-E1	LC3	Increased

Sambandam *et al.* (2014) studied the impact of microgravity on the osteoclasts and proved that exposure in modelled microgravity for 24 h resulted in increased osteoclastogenesis and expression of autophagy markers Atg5 and LC3II in RAW264.7 cell culture as well as in mouse bone marrow cells (Sambandam *et al.*, 2014). Exposure of seminoma TCam-2 cells to modeled microgravity resulted in temporary changes of cytoskeleton and autophagy induction. More specifically, expression of LC3II, as estimated with Western blot analysis, was increased after 24 h of exposure to modeled microgravity and then was restored to the basic level after 48 h, indicating a temporary and adaptive role of autophagy in seminoma cells under microgravity conditions (Ferranti *et al.*, 2014). Indo *et al.* (2015) found no significant difference in BECN1 expression, which is an autophagy marker, among neuroblastoma cell line NB-1 cultured under modeled microgravity conditions for 24 h and the same cell culture under earth gravity conditions (Indo *et al.*, 2015).

Human embryonic renal cell line HEK293, which expresses GFP-LC3 constantly, had increased numbers of autophagosomes after 72 h, but not after 24 h, of exposure to modeled microgravity and elevated levels of phosphorylated protein kinase dependent on AMP (AMPK). Suppression of AMPK expression resulted in the restoration of autophagy markers to comparable levels to the reference cell culture, indicating the involvement of AMPK in the induction of autophagy pathway, but not as a unique regulator (Ryu *et al.*, 2014). The effects of autophagy and microgravity are summarized in Table 3.

Microgravity, autophagy and osteoclastogenesis

Microgravity has detrimental effects on bone metabolism. Weight-bearing bones experience a significant decrease in bone density. Prolonged exposure to microgravity conditions results in increased bone resorption in the early stages and decreased bone formation later. Osteoclasts are responsible for bone resorption and play an important role under microgravity conditions. Autophagy is a mechanism which is activated under stress. Microgravity produces a stressful environment for the bone. Researchers, such as Sambandam *et al.* (2016), have studied the possible role of autophagy as the mediator of osteoclastogenesis under microgravity conditions (Sambandam *et al.*, 2014).

Preosteoclasts cultured under microgravity conditions expressed increased levels of Atg5, LC3 and Atg16L, which are autophagy markers. The researchers also observed abundant autophagosomes in RAW264.7 cells cultured under modelled microgravity conditions using confocal microscopy. Sambandam *et al.* observed increased expression of genes taking part in signalling pathways associated with autophagy, such as GAA, Trp53, Prkaa1, Rps6kb1, or being components of autophagosomes, such as Atg16L, Atg9b, Irgm1, using RT2 profiler polymerase chain reaction array method. They also noted a significant increase in expression of phospholipase PLCγ2 under microgravity conditions, whereas the signalling pathway of PLCγ2/calcium regulates autophagy in preosteoclasts (Sambandam *et al.*, 2014).

Administration of 3-methyladenine, which is an autophagy inhibitor, to mouse bone marrow cells suppressed the expression of Atg5 and LC3, which are autophagy markers, as well as cathepsin K, which is an osteoclast marker, indicating that autophagy probably plays a role in osteoclastogenesis under microgravity conditions (Sambandam *et al.*, 2014).

Although autophagy is a significant player in bone metabolism under microgravity conditions, one possible treatment would be the attempt to inhibit the autophagic machinery and thus alleviate relative outcomes. Yet, although this entails a large 'temptation', autophagy inhibition can have several effects on cellular physiology. As aforementioned, autophagy consists of a defensive mechanism of a dual nature. Autophagy is relatively at low levels, in mammalian cells and it is triggered by starvation and oxidative stress (Guo and White, 2016; Zhao *et al.*, 2016; Xu *et al.*, 2017), while it can function in a protective manner in the tumour microenvironment and promote tumour cell proliferation (Liu *et al.*, 2016*a*). Further on, it consists of a defense mechanism in DNA damage, which leads to controlled cell death (Roos *et al.*, 2016). On the other hand, numerous reports indicated the role of autophagy in malignant cell survival and proliferation. For example, it has been reported that autophagy inhibition has potential antitumor effects in glioblastoma (Zhang *et al.*, 2018), pancreatic adenocarcinoma (Boone *et al.*, 2018), bladder urothelial carcinoma (Zeng *et al.*, 2018), gastric cancer (Sun *et al.*, 2017), acute lymphoblastic leukemia (Lambrou *et al.*, 2012) and many others. It is evident that autophagy plays a significant role in cellular physiology as it consists of a protective mechanism under normal conditions and it is a facilitator of carcinogenesis and chemotherapy

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resistance under pathophysiological conditions. Thus, the mechanisms underlying those phenomena still remain to be elucidated and we still know very few for the complete role of autophagy in cellular pathophysiology. This leads us to the conclusion that the regulation of bone metabolism through autophagy inhibition could prove a risky alternative.

Autophagy and the astrobiological roadmap

Autophagy is one, among many, important cellular mechanism. Its understanding is crucial, both towards the elucidation of disease mechanics as well as cellular physiology. Yet, another emergent aspect of autophagy concerns space biology. As we have discussed in the previous sections, autophagy appears to be closely related to bone metabolism and is influenced by the presence (or absence of gravity). This indicates that autophagy, as many other cellular mechanisms, evolved together or due to the gravitational forces. This remark is directly connected to the third basic question of astrobiology and in addition to the fourth and sixth objectives, as stated in the NASA astrobiology roadmap, that is (a) the comprehension of how life on earth has co-evolved throughout geological time and (b) the understanding of the principles that will shape the future of life on earth and extraterrestrially (Des Marais *et al*., 2008). It will not be too long until the habitation of other planets will consist of a necessity for humanity, especially after what we humans have caused to our home. Space travel is one of the main challenges that should be dealt with since gravity has effects on the cardiovascular system, bone metabolism and to our opinion the immune system, since space is sterile and humans cannot survive without *their bacteria*.

Yet, musculoskeletal changes remain an immense challenge. Skeletal changes and loss of total body calcium have been reported in mammals (both humans and animals) exposed to microgravity from 7 to 237 days. In particular, photon absorptiometry measurements reported during the Apollo and Skylab missions showed that pre and post-flight bone mineral mass manifested an average post-flight loss from the *os calcis* (heel) of 3.2% over an average of 8.5 days (Hughes-Fulford, 2002). Analysis of in-flight urine, fecal and plasma samples from Skylab missions revealed changes in the urinary output of hydroxy-proline indicating degradation of the collagenous matrix substance of weight bearing bones (Hughes-Fulford, 2002). Elevated concentrations of urinary calcium were noted in the early studies of Skylab astronauts starting during the first days of flight. In many of the astronauts, urinary calcium concentrations remained at elevated levels throughout the mission. These physiological changes consisted of a direct effect of microgravity on the skeletal system, due to mechanical stress. More interestingly, these changes took place despite in-flight exercise, which was regularly performed. These observations manifested the significance of gravity and not of sole force application on the musculoskeletal system. It is probable that force by itself it is not adequate to stimulate the bone-forming/preserving machinery and the presence of the gravitational *field* is imperative.

Even though there are plans for a manned mission to Mars, the difference of the gravitational forces between Earth and Mars would create a series of physiological changes that are difficult to fully anticipate.

From the knowledge gathered so far, it has been found that almost the complete mammalian gene regulatory machinery is affected by the absence of gravitation. In a recent report, it has been found that osteoblasts are affected by microgravity as they manifest distinctive expressional profiles under the different gravitational conditions (Hu *et al*., 2017).

All the aforementioned questions and remarks are of major concern to the future of life, especially of extraterrestrial life. Bone metabolic machinery and along with it autophagy, is at the centre of the problem that makes human extraterrestrial habitation extremely challenging.

Discussion

Microgravity experienced by living organisms during spaceflights causes significant alterations in physiological functions. Thus, research has focused its attention on the possible underlying pathophysiological mechanisms in order to develop countermeasures to protect the human body from potential hazards faced in space. It has been observed that microgravity tends to increase autophagy, but this has not been confirmed by all studies, as some of them did not showe any difference between autophagy levels under microgravity conditions and under earth gravity circumstances.

It has to be mentioned that the results of different studies are not directly comparable, because different researchers have studied different types of cells. Different methods have been used in order to reproduce microgravity, including real microgravity during spaceflights, rotator wall vessels, animal and human analogs to microgravity, indicating a variance in microgravity level achieved in different experiments. In addition, the duration of exposure to microgravity ranges between 24, 48, 72 h for cell cultures up to 90 days for organisms and cell cultures loaded on actual spaceflights. Even though most researchers have studied the changes of LC3II, as an autophagy marker, under microgravity conditions, some researchers have used other autophagy markers, such as Atg5, Beclin-1 and p62.

The impact of autophagy on osteoclastogenesis has been studied by several researchers, who have conducted the conclusion that autophagy induces osteoclastogenesis. Experiments using inhibitors of autophagy, such as chloroquine and isoliquiritigenin resulted in suppression of osteoclastogenesis, indicating a possible role of autophagy inhibitors in the treatment of bone loss in the future (Xiu *et al*., 2014; Liu *et al*., 2016*b*). In addition, induction of autophagy in osteocytes resulted in a reduction of interferon-β, which in turn increased osteoclastogenesis, demonstrating the role of osteocytes in osteoclast maturation (Wang *et al*., 2017).

Autophagy is an attractive mechanism which mediates the effects of microgravity on osteoclastogenesis. Autophagy inhibitors, such as 3-methyladenine, reversed the increase in osteoclastogenesis caused by microgravity in mouse bone marrow cells, indicating a possible role in the treatment of bone loss astronauts face during spaceflight (Sambandam *et al*., 2014). In addition, TRAIL factor, which increases in preosteoclasts under microgravity conditions, induces autophagy in stem cells, supporting that autophagy could be a treatment candidate in order to eliminate bone loss caused by microgravity (Sambandam *et al*., 2016).

Conclusions

Microgravity conditions produce a rather hostile environment for the human body, with the bone loss being one of the most important consequences. Autophagy, which is a recycling cell process induced by stressful environment, seems to be a tempting therapeutic target since its suppression resulted in downregulation of osteoclastogenesis. Yet, its non-specific inhibition could also

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pose dangers for the cellular physiological machinery. Further on, research on autophagy and its relation to bone metabolism is directly linked to the third basic astrobiological question on the future of life, as well as the objectives of the astrobiological research.

More research has to be done in order to reach a more definite conclusion about the role of autophagy in osteoclastogenesis under microgravity conditions.

Author contributions. IM collected literature, drafted the manuscript; GIL proof-read the manuscript, drafted the manuscript, provided a critical review and gave final permission for submission. All authors read and approved the final manuscript.

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