

UNISS
UNIVERSITÀ
DEGLI STUDI
DI SASSARI

UNIVERSITY OF SASSARI

DEPARTMENT OF BIOMEDICAL SCIENCES

PhD Course in Biomedical Science

Curriculum Biology and Genetics

Molecular and Morphological Features of Mesenchymal Stem Cells Under Microgravity

PhD Course Director:

Prof.ssa Margherita Maioli

Supervisor:
PhD Candidate:

Prof.ssa Margherita Maioli
Dott: Renzo Pala

Co-Supervisor:

Dott.ssa Sara Cruciani

Cycle XXXVII

Statement of co-authorship

The works described in this Thesis gave rise to original articles published in the: ***International Journal of Molecular Sciences https://doi.org/10.3390/ijms24097753***

Renzo Pala: Methodology, Investigation, Software, Data Curation, Writing Original Draft Preparation

Sara Cruciani: Conceptualization, Methodology, Investigation, Software, Data Curation, Writing Original Draft Preparation

Alessia Manca: Methodology

Giuseppe Garroni: Methodology, Investigation

Mohammed Amine EL Faqir: Methodology

Veronica Lentini: Methodology

Giampiero Capobianco: Resources

Antonella Pantaleo: Validation, Writing review and editing, Visualization

Margherita Maioli: Conceptualization, Validation, Investigation, Writing review and editing, Funding Acquisition,Supervision

Stem cells *2*
Stem Cell Classification *5*
Embryonic Stem Cells **5**
Adult stem cells **5**
Mesenchymal stem cells 6
Wharton's Jelly MSCs 8
Microgravity *10*
Microgravity and Cellular Interactions *11*
Cellular stress *12*
Cellular Stress Induced by Microgravity *13*
Role of srituins *15*
Heat shock protein *16*
Cytocrome c *17*
Cytosckeleton *18*
Aim of research *19*
Material and methods *20*
WJ-MSC Isolation and Culture **20**
Microgravity Simulation **21**
RNA Extraction and Quantitative Polymerase Chain Reaction **22**
Immunofluorescence Analysis **23**
Western Blot Analysis **23**
Statistical Analysis **24**
Results *24*
Discussion *32*
Conclusions *36*
References *37*

Stem cells

Stem cells are undifferentiated, primordial cells with the exceptional ability to differentiate into over 200 distinct cell types that make up the human body, including neurons, skin cells, muscle cells,

bone cells, and liver cells.[1] The three fundamental properties of stem cells are: (a) clonality, which refers to their ability to proliferate from a single progenitor cell, forming a homogeneous population of cells; (b) self-renewal, which is the ability to divide and generate an identical copy of themselves, allowing for the maintenance of an undifferentiated state over long periods of time and ensuring a constant supply of stem cells throughout an organism's life; and (c**)** potency, which defines their capacity to differentiate into a wide variety of specialized cell types, ranging from pluripotency, where a stem cell can become virtually any cell in the body, to multipotency, where stem cells differentiate into specific types of cells within a particular tissue or organ system. These properties enable stem cells to play critical roles in development, tissue repair, and regeneration.[2] Zygote and blastocyst are the earliest stages of human development from which embryonic stem cells (ESCs) are derived. These ESCs have the unique ability to differentiate into the three primary germ layers endoderm**,** mesoderm, and ectoderm which subsequently give rise to the various tissues and organs of the human body. The endoderm forms internal structures such as the lungs and gastrointestinal tract, the mesoderm gives rise to muscles, bones, and blood vessels, while the ectoderm develops into the nervous system, skin, and other external structures. In addition to these embryonic origins, progenitor cells located in various adult tissues, such as bone marrow, bone, blood, muscle, liver, brain, adipose tissue, skin, and the gastrointestinal tract, also contribute to the formation and regeneration of organs throughout an individual’s life. These progenitor cells, although capable of differentiating into specific cell types, do not fully differentiate into all cell types, thus retaining some degree of plasticity and allowing them to participate in ongoing tissue repair and regeneration.[3] Tissue stem cells, often referred to as progenitor cells, are responsible for generating the terminally differentiated and specialized cells of a particular tissue or organ. These cells remain in a quiescent or inactive state under normal conditions, maintaining the integrity of the tissue. However, when injury occurs, these progenitor cells are activated to proliferate and differentiate, playing a crucial role in tissue repair and regeneration. Through their ability to respond to damage, tissue stem cells ensure the continuous renewal and maintenance of the tissue, replenishing lost or damaged cells and restoring normal function. Despite their limited capacity for differentiation compared to embryonic stem cells, progenitor cells are vital for maintaining tissue homeostasis and promoting recovery after injury.[4] Tissue stem cells, also referred to as progenitor cells, exhibit distinct dynamics based on the specific tissue in which they reside. In certain tissues, such as the pancreas, heart, and nervous system, these stem cells primarily remain in a quiescent state but are activated to proliferate and replace damaged cells following injury. In contrast, in tissues like the bone marrow, liver, lung, and gastrointestinal tract, stem cells are actively involved in maintaining tissue homeostasis. These cells regularly proliferate to support normal turnover, replenishing specialized cells that are continuously lost due to natural wear and tear, or to respond to injury and facilitate tissue repair. The capacity for regular turnover and regenerative response highlights the crucial role of tissue-specific stem cells in both routine tissue maintenance and in more acute repair processes

following damage.[5] Several key regulatory genes, including octamer-binding transcription factor 4 (Oct-4), SOX2, and NANOG, play a pivotal role in governing the essential characteristics of stem cell pluripotency. These genes are crucial for maintaining the undifferentiated state of stem cells, enabling them to retain the ability to differentiate into a wide variety of cell types. Oct-4, SOX2, and NANOG form a transcriptional network that sustains pluripotency by regulating the expression of other genes involved in self-renewal and differentiation. This network has been extensively utilized in vitro for the generation of induced pluripotent stem cells (iPSCs), a breakthrough technology that reprograms differentiated somatic cells back into a pluripotent state, effectively "resetting" their developmental potential. Through the careful modulation of these regulatory genes, researchers have been able to generate stem cells with properties similar to embryonic stem cells, opening new avenues for regenerative medicine, disease modeling, and drug discovery.[6] By activating genes involved in the self-renewal program and inhibiting those responsible for differentiation, the stem cell factors Oct-4, SOX2, and NANOG form a critical triad of transcription factors that are indispensable for preserving the pluripotency and self-renewal capabilities of stem cells. These factors work in concert to ensure that the stem cells maintain an undifferentiated state, essentially safeguarding their ability to proliferate indefinitely without committing to a specific cell fate. The triad exerts control over a wide array of genetic loci, orchestrating a complex regulatory network that maintains the balance between self-renewal and differentiation. By precisely regulating the expression of thousands of genes, Oct-4, SOX2, and NANOG ensure that the genome of stem cells remains in a flexible, undifferentiated state, allowing them to differentiate into various specialized cell types when appropriate, while also preserving the potential for continuous self-renewal. This dynamic regulation is essential not only for normal development but also for the successful generation of induced pluripotent stem cells (iPSCs) in laboratory settings. [7] It is well established that the expression of key pluripotency genes, such as Oct-4, SOX2, and NANOG, is progressively downregulated as stem cells age and undergo senescence. This reduction in gene expression significantly impacts the stem cells' ability to maintain their undifferentiated state and self-renewal capacity. As these genes become less active, stem cells lose their ability to efficiently switch on specific cellular phenotypes, which is essential for tissue regeneration and repair. The downregulation of these pluripotency factors is a key characteristic of aging stem cells and contributes to the decline in their regenerative potential. In senescent stem cells, this dysregulation impairs their ability to respond to cues for differentiation and tissue repair, leading to a reduced capacity for tissue regeneration and an increased susceptibility to age-related diseases. Consequently, understanding how aging and senescence affect the regulation of these key genes is critical for developing strategies to rejuvenate aging stem cells and improve their therapeutic potential.[8]

Stem Cell Classification

Based on where they came from, stem cells can be divided into four major groups: ESCs, fetal and adult stem cells, and iPSCs.[9]

Embryonic Stem Cells

Pluripotent embryonic stem cells (ESCs) are generated from the inner cell mass of the blastocyst, a stage of the pre-implantation embryo that occurs 5-7 days after fertilization [10] These cells have the ability to differentiate into the tissue of the three basic germ layers, but they can also be kept in culture for an extended amount of time without differentiating.[11] There are two layers of cells in a blastocyst: the trophoblast (outer cell mass), which forms the placenta, and the inner cell mass, which becomes the embryo.[12] To sustain the pluripotent state, ESCs depend on a complex network of transcription factors, of which Nanog, Oct4, and Sox2 are the key players.[13] Oct4 (Pou5f1), a POU-domain protein, counteracts tropoectoderm (TE) differentiation to specify the inner cell mass (ICM) during embryonic development, in fact, maintaining the pluripotent state in vitro requires carefully regulated amounts of Oct4.[14] Oct4 overexpression induces mesoderm (ME) and endoderm (EN) differentiation, Oct4 inactivation in ESCs results in a rapid loss of self-renewal and induction of (TE) differentiation.[15] The homeodomain of the Nanog protein regulates the choice made by the blastocyst between epiblast and primitive endoderm (pEN); in fact, in vitro, blastocysts devoid of Nanog are unable to form ESCs or differentiate into pEN lines.[16] Downregulation of Nanog in ESCs can give rise to a broader spectrum of cell lines, with a more marked contribution toward TE and pEN [15] Sox2, an HMG box protein linked to SRY, is necessary for the maintenance of epiblasts, and mESCs depleted of Sox2 differentiate into TE.[17] Molecular investigations in embryonic stem cells have demonstrated that Nanog, Oct4, and Sox2 co-occupy several regulatory loci and coregulate vast gene cohorts, maintaining the regulatory network accountable for pluripotency and self-renewal.[18] These proteins facilitate gene silence when they bind to developmental regulator promoters, but they also engage the global transcriptional coactivator p300 when they attach to active enhancers. [19]

Adult stem cells

The human embryonic stem cells are isolated from the blastocyst only after the embryos are killed, thus using them has ethical implications. Scientists became interested in somatic stem cells because of these ethical questions.[20] Adult stem cells, also known as somatic stem cells (ASCs), can develop into a particular phenotype based on the characteristics of mature cells in the tissue in which they reside. When compared to embryonic cells, they have lower telomerase activity levels and a restricted capacity for differentiation.[21] In fact, adult stem cells differentiate gradually toward the mature state as part of an irreversible senescence process. These factors make them multipotent stem cells.[22] Every tissue in the body has been shown to be a source of stem cells that can be

employed in autologous transplants to enable the development of mature phenotypes.[23] For instance, bone marrow-derived stem cells (BM-MSCs) have the ability to develop into bone, glia, skeletal muscle, and hematopoietic line cells.[24] Adipose tissue, cartilage, and skeletal muscle can all be differentiated from dermal stem cells. The "plasticity" of adult stem cells has been characterized as their capacity to form distinct lineages.[25] Even if their pluripotency is not as high as that of embryonic stem cells, their degree of flexibility indicates that they may be used in the treatment of numerous disorders.[26] Hematopoietic stem cells (HSC) are the first fully described adult cells. They facilitate hematopoiesis, which produces all blood components.[27] Based on the antigenic features of their surface, they were initially isolated in mice: they express CD45, Thy1, c-kit, and Sca-1, but not lineage (Lin) and CD34 antigens.[28] Unlike mouse antigens, human HSCs express CD45, c-kit, and Thy1, and they are found in the CD34+ fraction of bone marrow, peripheral blood, and cord blood. However, human HSCs do not express lineage (Lin) or CD38 antigens.[28] These criteria have recently been used to distinguish adult stem cells from different sources, such as neural stem cells, which are distinguished by a combination of CD133 and CD24.[29]

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a type of adult stem cells that have garnered substantial interest in regenerative medicine, owing to their remarkable ability to differentiate into a variety of cell types and their potential to support tissue repair and regeneration. These cells are predominantly isolated from a range of adult tissues, such as bone marrow, adipose (fat) tissue, umbilical cord blood, and dental pulp, among others. Their multipotency allows them to differentiate into various specialized cell types, including osteocytes (bone cells), adipocytes (fat cells), chondrocytes (cartilage cells), and others, making them invaluable in therapeutic applications for conditions involving tissue damage or degeneration. MSCs also exhibit immunomodulatory properties, which further enhance their utility in treating inflammatory diseases and facilitating tissue graft acceptance. Their capacity to promote healing and tissue regeneration has sparked their exploration in clinical trials for a wide range of medical conditions, from orthopedic injuries to cardiovascular diseases and neurodegenerative disorders. [30] Mesenchymal stem cells (MSCs) exhibit an extraordinary capacity to differentiate into a wide range of specialized cell types, such as osteoblasts (bone-forming cells), chondrocytes (cartilage cells), adipocytes (fat cells), and even cardiomyocytes (heart muscle cells) when provided with the appropriate conditions. [31] This multilineage differentiation potential underscores their significant role in tissue regeneration and repair. Beyond their ability to transform into various cell types, MSCs are also renowned for their immunomodulatory properties. [32] They possess the unique ability to regulate immune responses, which makes them particularly valuable in treating immune-related disorders. This includes conditions such as graft-versus-host disease, where the immune system attacks transplanted tissues, as well as other autoimmune and inflammatory diseases. The dual capability of MSCs to promote tissue regeneration while simultaneously modulating immune reactions makes them a promising therapeutic tool in a variety

of clinical settings, including organ transplantation, autoimmune disorders, and chronic inflammation. [33] Mesenchymal stem cells (MSCs) are recognized for their ability to secrete a wide array of bioactive molecules, including cytokines, growth factors, and extracellular matrix components, which are pivotal to their regenerative capabilities. These secreted paracrine factors are thought to exert trophic effects on damaged tissues, promoting cell survival, proliferation, and differentiation. [34] For example, MSCs secrete key growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor 1 (IGF-1), all of which have been shown to play a crucial role in supporting tissue repair in various tissues, including bone, cartilage, muscle, and even neural tissue. [35] Moreover, MSCs have been found to promote angiogenesis, the formation of new blood vessels, reduce fibrosis (the formation of excessive scar tissue), and enhance wound healing, thereby contributing to the restoration of tissue function after injury. [36] These regenerative properties have made MSCs a promising therapeutic approach in both preclinical and clinical trials aimed at treating a broad spectrum of conditions, such as osteoarthritis, myocardial infarction (heart attack), and spinal cord injury. [37] As the understanding of MSCs' molecular mechanisms continues to evolve, their potential applications in regenerative medicine are expanding, offering new hope for patients suffering from various degenerative and traumatic conditions. The therapeutic potential of mesenchymal stem cells (MSCs) extends well beyond immune modulation and tissue regeneration. MSCs have demonstrated significant promise in the treatment of bone and cartilage defects, primarily due to their osteogenic (bone-forming) and chondrogenic (cartilage-forming) differentiation capabilities. For instance, bone marrow-derived MSCs (BM-MSCs) have been used in clinical studies for bone tissue engineering, where they have shown the ability to promote bone formation and aid in the repair of bone defects. [38] This has led to their consideration as a viable option for treating conditions like bone fractures, osteoporosis, and other skeletal disorders. In cartilage repair, MSC-based therapies have proven effective in treating ailments such as osteoarthritis and cartilage defects. MSCs can differentiate into chondrocytes (cartilage cells) and produce cartilage-like tissue, thus offering a potential solution for regenerating damaged cartilage and alleviating pain and disability associated with joint degeneration. [39] This regenerative approach has been explored as a means to restore joint function and slow the progression of degenerative diseases. Additionally, MSCs are being investigated for their potential in cardiac repair. Preclinical studies have revealed that MSCs can either differentiate into cardiomyocytes (heart muscle cells) or contribute to tissue repair by secreting growth factors that stimulate angiogenesis (formation of new blood vessels) and enhance cardiac tissue regeneration after a myocardial infarction (heart attack). [40] This opens up exciting possibilities for using MSC-based therapies to treat heart disease and improve recovery following cardiac events. As research continues, the versatility of MSCs in addressing a wide range of tissue damage and degenerative conditions positions them as a key player in advancing regenerative medicine.

Wharton's Jelly MSCs

The human umbilical cord, which connects the growing embryo or fetus to the placenta and weighs approximately 40 g, is 3065 cm long and has an average diameter of 1.5 cm. It serves as a link between the mother and the fetus during pregnancy. [41] It begins to form from the yolk sac and allantois on day 26 of pregnancy, and its primary roles include supplying the fetus with blood and eliminating biological waste. [42] In general, mesenchymal stromal cells (MSCs) can be divided into two groups: those originating from adult tissues like bone marrow or adipose tissue, and those originating from fetal or perinatal tissues like the placenta, umbilical cord, Wharton's jelly (WJ), amniotic membrane, etc.[43] Within the umbilical cord, Wharton's jelly forms the main connective tissue, it contains mesenchymal stromal cells (MSCs), which Thomas Wharton first described in 1656.[44] WJ-derived stem cells are a useful model of multipotent stem cells that are readily obtained from "wasting materials" without causing any ethical issues. [45] These cells have a younger phenotype and more plasticity in comparison to mesenchymal stem cells (MSCs) generated from bone marrow or adipose tissue.[46] They also originate from protected newborn tissue that had less external interference, as tissues might be affected by history of illness and lifestyle; this characteristic helps in the development of a more homogeneous cell cohort. [47] Although WJ-MSC are resistant to aging and lifelong exposure to environmental variables, they are less likely to develop faulty functionalities that can accumulate over the course of a cell's lifespan.[48] In fact, aging causes a reduction in activities and functions of tissue-derived MSCs, which poses serious challenges for MSC-based therapies in regenerative medicine.[49] Furthermore, even after differentiating into many lineages, WJ-MSCs retain their morphology and immunoprivileged condition for extended periods of time following in vitro passages. [50] It's crucial to remember that quality control for these cells should adhere to certain standards, like choosing samples from healthy donors with full-term pregnancies, women who are at least eighteen years old, water broken for no more than eighteen hours, and expectant mothers who have had at least two prenatal consultations and who do not exhibit fever or infection at delivery.[51] Hemoglobin electrophoresis and serology for common viruses and parasites should be part of the maternal serum screening prior to birth. According to multiple findings, MSC are immune privileged or hypoimmunogenic cells, a state that is probably bolstered by the placenta and the fetus, two nearby immunologically protected areas.[51] WJ-MSC lack the expression of MHC class II molecules and have low levels of MHC class I and of costimulators CD40, CD80, and CD86. [52] They show elevated production of immune suppression proteins, including prostaglandin E2 (PGE2), indoleamine-2,3-dioxygenase (IDO), and leukocyte antigen G6 (HLA-G6), which is crucial in inhibiting immunological reactions against the embryo.[53] It is essential to keep in mind that therapeutic applications employing MSC typically use hundreds of millions of cells per treatment and necessitate an initial *in vitro* expansion stage before their usage. Multiple passages in vitro have been demonstrated to increase senescence markers and impair BM-MSC self-renewal potential as indicated by telomere length shortening. [54] Only early passages are typically used in studies to

assess the immunomodulatory potential of MSC from various sources, and little information is now available on how they behave after passing *in vitro* until sufficient numbers of cells are obtained for use in cell therapy.[55] Despite differences in gene expression, one study comparing AD-MSC and BM-MSC from passages 4 and 10 revealed that they had similar cell shape, surface marker expression, and immunomodulatory characteristics.[56] Even while WJ-MSC has a longer lifespan in vitro, it will eventually cause cell arrest and replicative senescence, which will impair stem cell activity even though the senescent cells survive.[57] According to the literature, the lifespans of WJ-MSCs derived from various donors varied depending on the number of passages, growth potential, and senescent phenotype. [47] Furthermore, every WJ-MSC sample behaves differently, displaying different immunomodulatory properties and cytokine mRNA expression patterns. In fact, it is thought that meticulous assessment of senescence markers following multiple passages and surveillance of each collected cell's immunosuppressive potential ought to be part of quality control prior to therapeutic use.[58] Proteins known as embryonic stem cell markers, including Oct-4, Sox-2, Nanog, Rex-1 (Zinc finger protein 42), SSEA-1, SSEA-4, and nucleostemin, are expressed by the umbilical stroma and are thought to indicate pluripotency and self-renewal in primitive stem cells. [59] Additionally noted were the expression of adhesion molecules like CD44 or CD146, integrin markers like CD29 or CD51 [61], and CD24 and CD108, which are thought to verify the presence of MSCs.[60] MSC markers such CD29, CD44, CD73, CD90, and CD105 were positively expressed by Wharton's gelatin cells; these cells are also known to display the key histocompatibility marker class I, HLA-ABC, and lack the expression of the class II marker, HLA-DR3.[61] However, WJ-MSCs are prospective candidates for both allogeneic and xenogeneic transplantation since they do not express HLA-DR (MHC-II).[62] The immunomodulatory capabilities of these cells were initially reported *in vitro* by Weiss et al.; they showed that these cells do not promote the proliferation of allogeneic or xenogeneic immune cells, but rather inhibit the growth of human peripheral blood mononuclear cells and rat splenocytes. [53] Additionally, WJ-MSCs do not express immune response-related antigens that are implicated in T lymphocyte activation, such as CD40, CD80, or CD86, but they do create HLA-G6, an immunosuppressive form of human leukocyte antigen that inhibits the cytolytic activity of NK cells.[53] Since this effect was shown when WJ-MSCs were co-cultured with immune cells or when only the culture supernatant was employed, it was assumed that WJ-MSCs prevent these cells from proliferating through both soluble factors and direct cell-to-cell contact. Additionally, following co-cultivation, lymphocytes secreted less TGF-β1 (transforming growth factor β1) and IFN-γ (interferon γ).[63] Whereas Karaöz et al. showed that following WJ-MSC and T cell co-culture, pro-apoptotic genes like BCL2L10 (Bcl-2-like protein 10), BAK1 (Bcl-2-like protein 7), and BIK (Bcl-2-interacting killer) were upregulated while anti-apoptotic genes like BCL-2 and BIRC3 (Baculoviral IAP repeat containing 3) were simultaneously downregulated. This suggests that WJ-MSCs have apoptotic properties towards activated T cells.[64] According to Deng et al. mesenchymal stem cells isolated from the umbilical cord release IL-6 (interleukin 6), which allows

them to direct dendritic cells to develop tolerogenic phenotypes.[65] MSCs also release other soluble molecules that are involved in immunoregulation, such as galectin-1, HLA-G5, PGE2 (prostaglandin E2), TGF-β1, IL-10 (interleukin 10), HGF (hepatocyte growth factor), and IDO (indoleamine 2,3-dioxygenase).[53] In addition, it has been reported that WJ-MSCs exhibit upregulated expression of anti-inflammatory molecules such as CD200 or PD-L1 (Programmed death ligand 1).[66] Furthermore, Huang et al. successfully differentiated WJ-MSCs into germ-like cells, and this was verified by the expression of markers characteristic to germ cells, including Oct4, CD117 (C-kit), and CD49f.[67]

Microgravity

The clinostat is a device designed to simulate microgravity conditions by continuously rotating biological samples around one or more axes, thereby nullifying the directional influence of gravity over time. [68] The fundamental principle behind clinostat operations is the constant reorientation of the gravity vector relative to the sample, which prevents cells and tissues from perceiving a consistent gravitational stimulus. This rotation can be achieved using either a single-axis or a dual-axis clinostat, with the latter providing a more uniform distribution of the simulated microgravity effect. [69] Clinostats have been extensively used in plant biology, cell biology, and developmental studies to investigate the effects of altered gravitational conditions on growth, differentiation, and gene expression. In plant research, clinostats help elucidate the mechanisms of gravitropism by observing how root and shoot orientations change in the absence of a fixed gravity vector. [70] In cell biology, they are employed to study cytoskeletal organization, cell signaling pathways, and the behavior of various cell types under microgravity-like conditions, which has implications for space biology and regenerative medicine[71] . Moreover, clinostats play a crucial role in space-related research, serving as ground-based analogs to actual microgravity environments experienced in space missions. They provide a cost-effective and accessible means to conduct preliminary experiments before spaceflight studies, aiding in the development of countermeasures for space-related health issues such as muscle atrophy and bone density loss. [72] The clinostat is an essential tool for simulating microgravity conditions in cell culture studies, widely utilized in gravitational biology to investigate the effects of reduced gravity on cellular behavior. By continuously rotating samples around a horizontal or multi-axial axis, the clinostat effectively averages the gravitational vector over time, thereby minimizing sedimentation and the influence of unidirectional gravitational forces. [71] This dynamic environment alters the mechanical stimuli perceived by cells, allowing researchers to study how microgravity impacts fundamental biological processes such as cell proliferation, differentiation, apoptosis, gene expression, and cytoskeletal organization. [73] Clinostat-based experiments have demonstrated significant alterations in cellular morphology, with notable changes in actin filament organization, extracellular matrix production, and signal transduction pathways. [74] In stem cell research, the clinostat has been instrumental in revealing the role of mechanical forces in lineage

commitment and differentiation potential, highlighting how microgravity-like conditions can promote undifferentiated states or favor specific differentiation pathways. [75] Additionally, studies on immune cells have shown that simulated microgravity can impair T-cell activation and cytokine production, suggesting potential risks for immune dysregulation during long-term space missions. [76] In cancer biology, clinostat experiments have provided insights into how microgravity influences tumor cell growth, metastasis, and resistance to chemotherapy, offering new perspectives for cancer research both in space and on Earth. [77] Despite its effectiveness, the clinostat has limitations, such as the inability to fully replicate the complex fluid dynamics and mechanical unloading of true microgravity environments encountered in space. [72] Nevertheless, due to its cost-effectiveness, ease of use, and adaptability for different experimental setups, the clinostat remains a valuable platform for preliminary studies prior to spaceflight experiments, contributing significantly to our understanding of gravity-related biological mechanisms.[78]

Microgravity and Cellular Interactions

Microgravity, the condition of near weightlessness experienced in space, exerts a significant influence on cellular behavior, impacting various critical processes such as cell morphology, proliferation, differentiation, and gene expression. In the absence of gravitational forces, cells undergo alterations in shape and structure, which can affect their ability to maintain normal functions. Research has shown that microgravity can disrupt normal cellular proliferation, leading to a decrease in cell division rates. Studies have indicated that exposure to microgravity impairs the proliferation of mouse embryonic stem cells, triggering processes such as senescence and apoptosis. These findings suggest that microgravity may act as a stressor that induces cellular aging mechanisms, including the activation of pathways responsible for programmed cell death. Moreover, microgravity's effects on gene expression have been highlighted, with alterations in the regulation of genes associated with cell cycle progression, stress response, and survival. Collectively, these observations underscore the profound impact of microgravity on stem cell behavior, with potential implications for tissue regeneration, cellular function, and the long-term health of astronauts during space missions.[79] In addition, microgravity has been shown to impact mitochondrial function, resulting in an enhanced production of reactive oxygen species (ROS). These ROS can lead to oxidative damage within cells. This oxidative stress has the potential to alter cellular aging and overall cellular functions. A review was conducted with the goal of investigating the underlying mechanisms by which microgravity triggers oxidative damage in mitochondria. The review focused on analyzing the expression of various genes and proteins, as well as examining the relevant metabolic pathways involved in this process.[80] In the context of stem cells, microgravity has been found to affect differentiation pathways. Specifically, studies have shown that exposure to microgravity can enhance the myocardial differentiation of induced pluripotent stem cells (iPSCs). This suggests that microgravity may play a role in modulating the differentiation process of stem cells, potentially

influencing the development of specialized cell types like cardiac cells.[77] Furthermore, microgravity impacts cell adhesion properties, which are essential for maintaining tissue integrity and function. Alterations in cell adhesion behaviors can disrupt critical processes such as cell-cell recognition, migration, and signal transduction each of which plays a key role in sustaining cellular homeostasis. The effects of microgravity extend to various physiological systems, including the cardiovascular, nervous, and immune systems, as well as bone tissue. These changes are, in part, driven by modifications in cell adhesion properties, which can contribute to the physiological adaptations and challenges observed in microgravity environments. [76] Understanding how cells respond to microgravity is crucial, particularly with the potential for long-duration space missions. The effects observed in microgravity offer valuable insights into fundamental cellular processes and mechanisms, which can enhance our knowledge of human health. These findings not only have significant implications for astronaut health during space travel but also provide important perspectives on various biological phenomena on Earth. By studying cellular behavior in microgravity, we can better understand aging, disease mechanisms, and other physiological changes that occur in altered gravity environments, offering potential applications for medical advancements and improved treatments for conditions on Earth. [81]

Cellular stress

An imbalanced redox state known as oxidative stress is defined by an excessive buildup of reactive oxygen species (ROS) or inadequate antioxidant defenses. [82] Acute ischemic cardiovascular (such as myocardial infarction) and cerebrovascular (such as stroke) diseases, Alzheimer's disease, parkinsonism, immunological disorders, and cancer are all influenced by oxidative stress.[83] ROS are ions or molecules that react chemically and are produced as byproducts of the metabolism of oxygen. They include singlet oxygen ($^1O_2$), hydroxyl radicals (•OH), hydrogen peroxide (H2O2), superoxide (O2•−) and lipid hydroperoxides (LOOH). At low concentrations, ROS act as signaling molecules that mediate gene expression, motility, differentiation, and cell proliferation. At larger doses, however, they negatively affect cell viability and function.[84] Superoxide dismutase 2 (SOD2) in the mitochondrial matrix and superoxide dismutase 1 (SOD1) in the mitochondrial intermembrane space and cytoplasm immediately convert O2− to more stable H2O2. [85] By immediately oxidizing certain sulfur-containing amino acids (such as cysteine and methionine), which are essential for a protein's stability, activities, and subcellular location, H2O2 acts as a signaling molecule. [86] Antioxidants can typically reverse these oxidations, and peroxiredoxins are the primary detoxifiers of H2O2 to water.[86] When ferrous iron (Fe2+) is present, H2O2 can also produce •OH. •OH and polyunsaturated fatty acids (PUFAs) combine to form LOOH, which can start ferroptosis.[87] LOOH can be detoxified by reducing ubiquinone (Q) to ubiquinol (QH2), which is dependent on glutathione peroxidase 4 (GPX4) and ferroptosis suppressor protein 1 (FSP1).[88] Different transcription factors, such as members of the forkhead box protein O (FOXO) family and nuclear factor (erythroid-derived

2)-like 2 (NRF2), control the expression of the antioxidant genes to keep ROS levels from rising to a point where stem cells would be harmed.[89] Our previous research has shown that the onset of cellular senescence can be modulated through the application of natural bioactive molecules, such as melatonin, which exhibit senescence-delaying properties.[90] In almost all of cell types, mitochondria are the main generator of intracellular ROS. Intracellular ROS generation is mediated by cytochrome P450 enzymes, cyclooxygenases, nitric oxide synthase, xanthine oxidase, NADPH oxidases, and other cell organelles such peroxisomes and the endoplasmic reticulum.[91] Understanding how ROS affect stem cells, particularly during oxidative stress, is crucial since a balanced redox status is necessary for cell maintenance and functioning, including in stem and progenitor cells.[85] Cellular detoxification systems can contribute to this condition by protecting cells physically and using the longevity factor, Sirtuin deacetylase family as well as HSP60, HSP70 and NOX4 to respond to stressors such as excessive levels of ROS.[92]

Cellular Stress Induced by Microgravity

One of the major concerns associated with prolonged exposure to microgravity is the cellular stress induced by the significant alterations in the environment. In a microgravity setting, cells face a variety of stressors that can severely impact their function and overall survival. These include oxidative stress, mechanical stress, and DNA damage. Specifically, microgravity leads to an imbalance in cellular homeostasis, resulting in an elevated production of reactive oxygen species (ROS). ROS are highly reactive molecules that can damage critical cellular structures, including proteins, lipids, and DNA. This oxidative damage disrupts normal cellular processes, impairing cell function and potentially triggering cellular apoptosis or senescence. Additionally, the mechanical stress in microgravity, caused by changes in fluid dynamics and cellular architecture, further exacerbates the risk of cell injury. The combined effects of oxidative stress, mechanical stress, and DNA damage create a complex environment where cellular integrity is constantly compromised, posing significant challenges for long-term human space exploration.[93] This stress also exerts a significant and profound impact on stem cells. Oxidative stress, which is exacerbated in microgravity, can disrupt the ability of stem cells to maintain their undifferentiated state, a crucial aspect for their regenerative potential. Under normal conditions, stem cells rely on a delicate balance of biochemical signals to remain undifferentiated and capable of differentiating into specialized cell types when needed. However, in the microgravity environment, this balance is disrupted, and the stem cell niche, which normally provides essential cues for self-renewal and differentiation, becomes altered. As a result, stem cells in microgravity are more susceptible to undergoing apoptosis (programmed cell death) or senescence (cellular aging), both of which severely compromise their regenerative capacity and limit their ability to repair tissue damage effectively. Moreover, oxidative stress in microgravity has been shown to induce DNA damage, which can cause mutations within stem cells. These mutations not only threaten the immediate functionality of the stem cells but may also jeopardize their long-term

viability, reducing their ability to function properly in tissue regeneration. Consequently, the compounded effects of oxidative stress, DNA damage, and disrupted stem cell niche contribute to a significant challenge for tissue repair and regeneration in space. [94] The mechanisms driving the cellular stress observed in microgravity are intricate and involve multiple factors. One of the primary effects of the absence of gravitational forces is the alteration of the mechanical properties of the cell membrane and cytoskeleton, both of which are essential for maintaining cellular shape, intracellular transport, and signal transduction. Under normal gravity conditions, the cytoskeleton plays a vital role in providing structural support to the cell, enabling it to respond effectively to external forces and internal signals. However, in microgravity, the disruption of the cytoskeleton impairs its normal function, leading to changes in the cell's ability to maintain its shape and respond to mechanical cues. This disruption has profound implications for how the cell reacts to both external and internal stimuli, which can include factors such as nutrient availability, mechanical forces, and biochemical signals. One critical consequence of cytoskeletal disruption in microgravity is the impairment of mechanotransduction pathways. These pathways are crucial for stem cell function, as they enable stem cells to sense and respond to mechanical signals, which are necessary for maintaining their undifferentiated state, motility, and differentiation potential. The dysfunction of mechanotransduction pathways under microgravity conditions can lead to alterations in cell morphology, affecting the shape and structure of stem cells. Additionally, stem cells may experience reduced motility, limiting their ability to migrate to areas of injury or tissue damage. Finally, the impaired mechanotransduction pathways compromise the stem cells' capacity for differentiation, which is essential for tissue repair and regeneration. As a result, the disruption of these cellular processes under microgravity can have significant consequences for stem cell behavior and overall cellular health. [95] Moreover, the changes in gene expression induced by microgravity play a significant role in the cellular stress response. Research has shown that microgravity leads to alterations in the expression of genes involved in crucial cellular processes, including apoptosis (programmed cell death), cell cycle regulation, and the oxidative stress response. These changes have been observed in both stem cells and differentiated cells exposed to microgravity conditions (Zhang et al., 2017). As a result of these genetic alterations, cells often experience an increase in the activation of pathways that can lead to detrimental outcomes such as cell death, senescence (cellular aging), or the dysregulation of stem cell differentiation. [96] Specifically, microgravity has been found to upregulate genes that are directly involved in the production of reactive oxygen species (ROS), which are highly reactive molecules that can cause oxidative damage to cellular components. The heightened ROS production is linked to the activation of cellular stress response pathways, such as the p53 and MAPK (mitogen-activated protein kinase) pathways, both of which play central roles in regulating cell survival, apoptosis, and stress responses. In particular, the activation of the p53 pathway, a well-known tumor suppressor and regulator of cell cycle arrest, apoptosis, and DNA repair, can lead to an increased risk of cellular damage, including the induction of cell death or senescence. Similarly, the MAPK pathway, which is

involved in various cellular processes, including growth, differentiation, and stress responses, can also be activated in response to the increased oxidative stress in microgravity. [97] These genetic and molecular changes in response to microgravity not only contribute to cellular dysfunction but can also have a profound impact on stem cell behavior. In particular, the dysregulation of stem cell differentiation and the increased vulnerability to apoptosis or senescence can limit the regenerative potential of stem cells in space, posing significant challenges to tissue repair and regeneration in a microgravity environment. [98]

Role of sirtuins

SIRT1 to SIRT7 are the seven members of the sirtuin family found in mammalian cells.[99] The SIRT protein attaches to a NAD+ molecule, cleaving nicotinamide and transferring the acetyl group from a substrate protein into the NAD+. This is a unique method for sirtuin deacetylation.[100] According to HPLC measurement, the end result is a 1:1:1 ratio of nicotinamide, O-acetyl-ADP-ribose, and the deacetylated substrate protein.[100] The catalysis of SIRT1 deacetylation activity requires NAD+ hydrolysis because without enzyme or substrate protein acetylation, no ADP-ribose or nicotinamide is produced from accessible NAD+.[100] Since sirtuins are linked to lifespan, interest in them has increased. A 30% increase in the life span of the yeast mother cell was seen when an additional copy of Sir2 converted into yeast led to a decrease in ribosomal DNA recombination.[101] The life duration of Caenorhabditis elegans was later found to be prolonged by the Sir2 homolog Sir2.1, indicating that the NAD+/NADH ratio and Sir2 homologs may likewise be in charge of prolonging the life span of higher eukaryotes like mammals.[102] Increased Sir2 levels continue to be associated with a 10%–14% increase in life expectancy, supporting the idea that SIRT1 plays a role in longevity.[103] SIRT1 has been shown to support cytoprotection when exposed to stressful situations, which is to be expected for a factor that can prolong life. [92] Numerous stress-induced transcription factors, such as p53, HSF1, NF-κB, PGC-1α, and the FOXO family of transcription factors, have been shown to be regulated by SIRT1's deacetylase activity. [104] By activating a number of stress response pathways, SIRT1 activation in response to stress may thus represent an evolutionarily conserved mechanism that promotes cellular homeostasis. [105] Reconnecting with the data already highlighted in our work, the tumor suppressor protein p53 induces the expression of p21 (WAF1/CIP1), a key regulator that hinders cell cycle progression by inhibiting cyclin-dependent kinase (cdk)/cyclin complexes, leading to G1/S phase arrest[106] Since SIRT1 is a crucial stress sensor, it follows that various cellular stresses can alter its activity and levels, enabling the proper cellular response to be coordinated.[107] The capability of sirtuins to degrade NAD+ during protein deacetylation, which produces nicotinamide and O-acetyl-ADP-ribose, links them to metabolism.[108] Metabolic stress is a key mechanism for controlling SIRT1 activity. SIRT1 is an energy sensor since it requires NAD+ as a crucial co-factor for enzymatic activity. SIRT1 activity may be modified as a result of dietary or metabolic abnormalities that alter NAD+, NADH, or their

ratio.[108] SIRT1 modulates several cell survival regulators, including as p53, FOXO, HSF1, NF-κB, and DNA damage response proteins, to improve cytoprotection under stress. [109] A well-known SIRT1 substrate, p53 plays a crucial role in tumor suppression, apoptosis, and cell cycle checkpoint regulation.[110] The acetylation status of p53 mediates its transcriptional activation through a number of protein kinases, while deacetylation decreases activity, enabling cells to evade death.[111] SIRT1 overexpression in cell lines improves cell survival following oxidative stress exposure and inhibited p53-dependent apoptosis.[110]

Heat shock proteins

The wide family of evolutionarily conserved molecular chaperones known as heat shock proteins (Hsps) plays essential functions in cell survival and development, based on similar molecular masses, they can be broadly divided into two families.[112] At first, the small, ATP-independent Hsps are chaperones with a molecular weight ranging from 8 to 28 kDa. Ubiquitin, the α-crystallins, and HspB1 (also called Hsp25 in mice or Hsp27 in rats and humans) are examples of these chaperones, in recent years, these tiny HSPs have drawn more attention, primarily because of their potential for early detection.[112] The functions of heat shock protein (HSP) molecules vary based on whether they are expressed intracellularly or extracellularly; they are primarily involved in the degradation and elimination of misfolded proteins, chaperone-mediated autophagy, protein quality control systems, cell signaling, the development of stress responses.[113] HSP60, HSP70, and HSP90 are the most well-researched and most conserved of the several families of HSPs; they are present in all organisms that have been investigated to so far and are in fact stress-inducible. [114] According to Parsell and Lindquist, the HSP60, HSP70, and HSP90 families are important for cell survival, stress management, and thermal tolerance in response to different types of heat shocks. [115] HSP60 is also referred to as phage growth E large (GroEL) in prokaryotes, such as bacteria, which have a unique ring-shaped structure or toroid quaternary structure that is found in eukaryotic mitochondrial cells. [116] In eukaryotes, HSP70 is known as HSC70 protein because of its distinct structure and constitutive expression of the homologous stress protein. [114] Our previous research demonstrated that increased HSP70 levels regulate T cell-mediated inflammation while protecting mitochondria and cells from apoptosis, and also play a crucial role in controlling autophagy during various physiological processes.[117] The molecular chaperones HSP60, 70, and 90 are crucial for intracellular organelles because they stabilize unstable proteins by controlling proper folding and assembling proteins into oligomeric shapes.[118] Additionally, these proteins play a role in a variety of biological functions, such as DNA replication, signal transduction, protein synthesis, and protein trafficking.[119]

Cytocrome c

Cytochrome c is a small heme protein located in the mitochondrial intermembrane space, playing a crucial role in the electron transport chain and cellular respiration by transferring electrons between Complex III (cytochrome bc1 complex) and Complex IV (cytochrome c oxidase)[120]. Structurally, cytochrome c contains a conserved heme-binding domain, which allows for its redox capabilities essential for ATP production. Beyond its bioenergetic functions, cytochrome c serves as a pivotal mediator of apoptosis, functioning as a key pro-apoptotic factor in the intrinsic pathway of programmed cell death.[120] Upon apoptotic stimuli such as DNA damage, oxidative stress, or growth factor deprivation, cytochrome c undergoes conformational changes that facilitate its release from the mitochondrial intermembrane space into the cytosol. [121] This release is a critical event in the intrinsic apoptotic pathway, as cytosolic cytochrome c binds to apoptotic protease activating factor-1 (Apaf-1) in the presence of dATP/ATP.[121] This interaction promotes the oligomerization of Apaf-1 into a heptameric structure known as the apoptosome, which recruits and activates procaspase-9. Activated caspase-9 subsequently triggers the caspase cascade, leading to the activation of effector caspases such as caspase-3 and caspase-7, ultimately resulting in the execution phase of apoptosis characterized by DNA fragmentation, membrane blebbing, and cell death [121]. The release of cytochrome c is tightly regulated by the Bcl-2 family proteins, which include both pro-apoptotic and anti-apoptotic members. [122] Pro-apoptotic proteins such as Bax and Bak promote mitochondrial outer membrane permeabilization (MOMP), facilitating the release of cytochrome c. Bax, in particular, undergoes conformational activation and oligomerization upon apoptotic signaling, forming pores in the mitochondrial outer membrane. [122] Conversely, anti-apoptotic proteins such as Bcl-2 and Bcl-xL inhibit MOMP by sequestering Bax and Bak, thus preserving mitochondrial integrity and preventing cytochrome c release [122]. The dynamic interplay between these Bcl-2 family members is modulated by BH3-only proteins which act as sensors of cellular stress and can either directly activate Bax/Bak or neutralize anti-apoptotic proteins. [120] This regulatory network ensures a tightly controlled balance between cell survival and apoptosis, with cytochrome c serving as a central effector in the execution of the apoptotic program. Furthermore, post-translational modifications of cytochrome c, such as phosphorylation, have been shown to influence its apoptotic potential, adding another layer of complexity to its regulation [120]. Emerging evidence also suggests that cytochrome c interacts with other cellular components beyond the canonical apoptosome pathway. For instance, cytochrome c can bind to cardiolipin, a phospholipid located in the inner mitochondrial membrane, which is crucial for maintaining mitochondrial structure and function. [123] During apoptosis, oxidative modification of cardiolipin facilitates the detachment of cytochrome c, promoting its release into the cytosol [124].

Additionally, cytochrome c has been implicated in redox signaling and the generation of reactive oxygen species (ROS), which can amplify apoptotic signals and contribute to cellular damage in pathological conditions such as neurodegenerative diseases and ischemia-reperfusion injury [125]

Cytosckeleton

The cytoskeleton is a highly dynamic and complex network of protein filaments, including microtubules, actin filaments, and intermediate filaments, that orchestrate a wide range of cellular functions. This intricate system not only provides structural support and maintains cell shape but also facilitates intracellular transport, mechano transduction, signal transduction, and plays an indispensable role in cell division, motility, and the spatial organization of organelles. [126] Microtubules, composed of α- and β-tubulin heterodimers, form rigid, hollow cylinders that serve as tracks for motor proteins like kinesin and dynein, enabling the directed movement of vesicles, organelles, and chromosomes during mitosis and meiosis. [127] Actin filaments, primarily composed of polymerized β-actin monomers, are flexible structures critical for generating cellular tension, enabling motility through lamellipodia and filopodia, and supporting the cell cortex to resist mechanical stress. Intermediate filaments, such as vimentin, keratins, and neurofilaments, provide tensile strength and structural resilience, anchoring organelles and maintaining the integrity of the nuclear envelope.[127] Alterations in the cytoskeletal architecture can lead to profound cellular dysfunctions. Disruptions in microtubule dynamics can impair mitotic spindle formation, leading to aneuploidy and contributing to oncogenesis. Defective actin polymerization and organization are associated with impaired cell migration, aberrant immune responses, and neurodegenerative diseases due to disrupted axonal transport. [127] The cytoskeleton's dynamic nature is tightly regulated through signaling pathways involving Rho GTPases, kinases, and actin-binding proteins, ensuring precise spatial and temporal control of cytoskeletal remodeling in response to extracellular cues.[127] The expression of genes encoding β-tubulin and β-actin is subject to stringent transcriptional and post-transcriptional regulation, reflecting their critical roles in maintaining cytoskeletal integrity and cellular adaptability. [74] Under microgravity conditions, such as those encountered during spaceflight, the absence of gravitational forces disrupts normal mechanical load and mechanotransduction pathways, leading to extensive cytoskeletal remodeling. This microgravity-induced alteration significantly affects the expression of β-tubulin and β-actin genes, resulting in disorganized microtubule networks, decreased microtubule stability, and disrupted actin filament architecture. These structural changes impair key cellular processes, including adhesion, migration, proliferation, and differentiation. [77] Experimental studies using simulated microgravity and spaceflight models have demonstrated that microgravity downregulates β-actin and β-tubulin mRNA and protein expression levels, leading to reduced cytoskeletal tension, altered focal adhesion dynamics, and compromised cell-cell and cell-matrix interactions. [128] Moreover, microgravity affects the post-translational modifications of cytoskeletal proteins, such as acetylation and

phosphorylation, which are crucial for their stability and function. These cytoskeletal alterations not only influence individual cell behavior but also affect tissue-level architecture and function, contributing to muscle atrophy, bone density loss, and immune system dysregulation observed in astronauts during prolonged space missions.[128] Understanding the regulatory mechanisms governing β-tubulin and β-actin gene expression under microgravity conditions provides valuable insights into cellular mechanobiology. It sheds light on how cells sense and adapt to changes in their physical environment, with broader implications for developing countermeasures to mitigate spaceflight-induced health risks. Additionally, these findings have translational relevance for terrestrial applications, including the design of biomaterials, tissue engineering, and regenerative medicine, where mechanical forces play a pivotal role in cell fate determination and tissue homeostasis.[129]

Aim of research

The aim of this study is to investigate the behavior of stem cells isolated from umbilical cord tissue when exposed to microgravity conditions, a unique and extreme environment that can significantly influence cellular physiology. Specifically, the research seeks to conduct an in-depth analysis of gene expression profiles associated with critical biological processes, including stemness, senescence, oxidative stress, apoptosis, and cytoskeletal modifications. Understanding the regulation of these genes under microgravity conditions is essential to elucidate how such an environment impacts stem cell function, viability, and differentiation potential. The study will focus on identifying key molecular pathways that are altered in response to microgravity, providing insights into the adaptive mechanisms that stem cells utilize in space-like conditions. This includes evaluating changes in the expression of pluripotency markers that define the self-renewal capabilities of stem cells, as well as genes involved in cellular aging and senescence, which may have long-term consequences on cell health and functionality. Furthermore, the research will explore how microgravity-induced oxidative stress influences the balance between cell survival and apoptosis, potentially altering the regenerative capacity of these cells. Given the critical role of cytoskeleton in maintaining cell structure, intracellular transport, and signal transduction, the study will also examine how microgravity affects cytoskeletal dynamics and organization. Alterations in the cytoskeletal architecture may lead to downstream effects on cell morphology, mechanical properties, and gene

expression patterns. Through comprehensive molecular and cellular analyses, this research aims to uncover potential changes in gene regulation and cellular behavior that could impact the application of stem cells in regenerative medicine, tissue engineering, and therapeutic interventions. Additionally, the findings could contribute to the development of strategies to mitigate the adverse effects of microgravity on human health, thus supporting the advancement of long-term space exploration and habitation

Material and methods

WJ-MSC Isolation and Culture

The study included umbilical cords (n = 6) obtained from healthy full-term women aged between 25 and 35 years, who were recruited based on the following criteria: spontaneous birth and no history of drug use, smoking, or diseases. The samples were collected after natural childbirth at the Gynecologic and Obstetric Clinic of the University of Sassari. Written informed consent was obtained from all participants, in accordance with the approval of the Ethics Committee (Ethical Clearance No. 0021565/2018, 22 March 2018, Commissione Etica CNR). All experiments were performed in duplicate (with three technical replicates), separately for each of the six samples. The umbilical cords were collected in phosphate-buffered saline (PBS) supplemented with 200 U/mL penicillin (Euroclone, Milano, Italy), 200 µg/mL streptomycin (Euroclone, Milano, Italy), and 4 mg/mL amphotericin B (Gibco Life Technologies), then stored at 4°C for subsequent WJ-MSC isolation. Tissues were dissected into small pieces and digested with collagenase type I (2 mg/mL) (Sigma Aldrich, Schnelldorf, Germany) at 37°C for 1 hour with continuous agitation. Following enzyme neutralization with 20% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA) and filtration through a 70 µm cell strainer (Euroclone, Milano, Italy), the samples were centrifuged at 600× g for 10 minutes. The resulting pellet was cultured in a basic medium (BM) composed of Dulbecco's modified Eagle's Medium (DMEM) (Life Technologies, Grand Island, NY, USA)

supplemented with 20% FBS (Life Technologies, Grand Island, NY, USA), 200 mM L-glutamine (Euroclone, Milano, Italy), and 200 U/mL penicillin–0.1 mg/mL streptomycin (Euroclone, Milano, Italy). The cell culture protocol was largely based on a previously established method, with certain adjustments made for particular cell types. [130] The cells were then cultured in T25 flasks at 37°C with 5% CO2 and saturated humidity for 10–14 days. [131] The culture medium was refreshed every 3 days. When cells reached 80–90% confluence, they were harvested using 0.25% Trypsin EDTA (Euroclone, Milano, Italy), counted, and transferred into new flasks. For immunomagnetic sorting, WJ-MSCs were selected for c-kit expression using a monoclonal anti-c-kit (CD117) antibody (Miltenyi Biotech, Minneapolis, MN, USA) conjugated directly to microbeads (Miltenyi Biotech). The WJ-MSCs utilized in this study were characterized by flow cytometry, as previously described. [132] For each treatment, after counting with the Tripan Blue and LUNA-II™ Automated Cell Counter, 5×10^5 cells/T25 flask were seeded, treated and then used for total RNA extraction. [98]

Microgravity Simulation

To investigate whether Wharton's jelly mesenchymal stem cells (WJ-MSCs) are affected by microgravity conditions, experiments were conducted using a 3D random positioning machine (RPM, Fokker Space, Netherlands) at the Department of Biomedical Sciences, University of Sassari, Sardinia, Italy. The 3D RPM is a microweight (microgravity) simulator based on the gravity vector-averaging principle, developed by Dutch Space. It consists of two perpendicular frames that rotate independently, continuously altering the direction of the gravity vector to simulate a microgravity environment. The 3D RPM generates simulated microgravity conditions of less than 10^{-3} g. The machine's dimensions are 1000 × 800 × 1000 mm (length × width × height). Connected to a computer, the RPM's mode and rotational speed were controlled using specific software, with Random Walk mode at 80 degrees/s (rpm) selected for this study. To replicate the effect of all operational conditions, the following procedure was applied. T12 flasks and chamber slides were carefully filled with WJ-MSCs in basic medium (BM), consisting of Dulbecco's modified Eagle's Medium (DMEM) (Life Technologies, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA), 200 mM L-glutamine (Euroclone, Milano, Italy), and 200 U/mL penicillin–0.1 mg/mL streptomycin (Euroclone, Milano, Italy), ensuring that no air bubbles were present to prevent fluid shear. The samples were kept at 37°C in a dedicated room during the experiment. Controls were placed in the static bar at 1 g to experience the same vibrations as the microgravity-exposed samples. Time points at 6, 12, 24, and 48 hours were selected for analysis. After the exposure, WJ-MSCs in the flasks were detached using 0.25% Trypsin EDTA

(Euroclone, Milano, Italy), centrifuged, and stored at −20°C for further characterization. For the chamber slides, samples were fixed in paraformaldehyde (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany). [98]

RNA Extraction and Quantitative Polymerase Chain Reaction

After treatment, total RNA was extracted using TRIzol® reagent and quantified by measuring absorbance at 260/280 nm with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 1 µg of total RNA was reverse transcribed into cDNA using the SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies, Grand Island, NY, USA). As previously described in our previous published papers [133], quantitative polymerase chain reactions (qPCR) were performed using a CFX thermal cycler (Bio-Rad, Hercules, CA, USA) under standard qRT-PCR conditions (50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, 55–59°C for 30 s, and 60°C for 1 min). The reactions were carried out using the Quantitative PCR Master Mix with Power SYBR® Green. For each reaction, 0.1 µM of each primer and 3 µL of cDNA (generated from 1 µg of total RNA) were mixed in a final volume of 25 µL. Ct values for the target genes were normalized against GAPDH [145], which was used as the reference gene. Gene expression levels in stem cells were calculated as fold change ($2^{-\Delta\Delta Ct}$) relative to the gene expression observed when the stem cells reached 80% confluence before treatment. A control with distilled water was included in each experiment. qRT-PCR analysis was performed on the following genes: Oct-4, SOX2, NANOG, SIRT1, p21 (WAF1/CIP1), p19 (ARF), p53, BAX, and Bcl-2. The primers used (Invitrogen) are listed in Table 1. [98]

Table 1. Primer sequences

Primer Name	Forward	Reverse
Oct-4	GAGGAGTCCCAGGCAATCAA	CATCGGCCTGTGTATATCCC
SOX2	CCGTTCATGTAGGTCTCGGAGCTG	CAACGGCAGCTACAGCTAGATGC
NANOG	CATGAGTGTGGATCCAGCT	CCTGAATAAGCAGATCCAT
P19	GCCTTCGGCTGACTGGCTGG	TCGTCCTCCAGAGTCGCCCG
P21 (WAF1/CIP)	CAAAGGCCCGCTCTACATCTT	AGGAACCTCCATTCACCCGA
P53	CAAGCAATGGATGATTTGATGCT	TGGGTCTTCAGTGAACCATTGT
BAX	TGCTTCAGGGTTTCATCCAG	GGCGGCAATCATCCTCTG
Bcl-2	AGGATTGTGGCCTTCTTTGA	ACAGTTCCACAAAGGCATCC
HSP70	CACAGCGACGTAGCAGCTCT	ATGTCGGTGGTGGGCATAGA
SIRT1	CATTTCCATGGCGCTGAGG	TGCTGGTGGAACAATTCCTGT
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG
P16	CTCGTGCTGATGCTACTGAGGA	GGTCGGCGCAGTTGGGCTCC
HSP60	GGGCATCTGTAACTCTGTCTT	TAAAAGGAAAAGGTGACAAGG

NOX4	GATGACTGGAAACCATACAAG	TAAAAGTTTCCACCGAGGACG
β-Actin	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT
β-Tubulin	CTGGACCGCATCTCTGTGTACT	GCCAAAAGGACCTGAGCGAACA

Immunofluorescence Analysis

Immunofluorescence analyses were carried out according to a protocol previously employed in earlier studies conducted by our research group [134]. WJ-MSCs (2.0 × 104 cells/ chamber slides) were incubated from 6 to 48 h under microgravity conditions (μg) and then fixed with 4% paraformaldehyde (Sigma Aldrich Chemie GmbH, Germany) for 30 min at room temperature. Control cells (Ctrl) were cultured under terrestrial (1 g) conditions. After fixation, cells were permeabilized using 0.1% Triton X-100 (Thermo Fisher Scientific, Grand Island, NY, USA)-PBS and then washed in PBS three times for 5 min. After washing, WJ-MSCs were incubated with 3% Bovine Serum Albumin (BSA) 0.1% Triton X-100 in PBS (Thermo Fisher Scientific, Grand Island, NY, USA) for 30 min and then exposed overnight at 4 °C to the primary monoclonal mouse cytochrome C (Cell Signalling , Danvers, Massachusetts, USA) primary antibody. After three washing steps in PBS for 5 min, cells were stained at 37 °C for 1 h in the dark with fluorescence-conjugated goat and mouse IgG secondary antibody (Life Technologies, USA). Nuclei were labelled with 1 μg/mL 4,6-diamidino-2-phenylindole (DAPI) (Thermo Fisher Scientific, Grand Island, NY, USA). All microscopy analyses were performed using a confocal microscope (TCS SP5, Leica, Nussloch, Germany). [98]

Western Blot Analysis

A previously established and validated immunoblotting protocol, as described in an earlier publication by our group, was employed for this analysis. [135] Cells were cultured under the previously described conditions for 6, 12, 24, and 48 hours in microgravity. Protein extraction was carried out using RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Grand Island, NY, USA; ref. 89900). For SDS-PAGE analysis, samples were thawed and incubated at 95°C for 5 minutes. The samples were then loaded onto a 10% polyacrylamide gel, subjected to electrophoresis, and transferred to nitrocellulose membranes for western blotting. To block nonspecific binding, membranes were incubated overnight at 4°C with gentle rocking in 5% non-fat milk–TBST [25 mM Tris, 140 mM NaCl, 3 mM KCl, 0.5% (v/v) Tween-20, pH 8.0]. Membranes were then probed with anti-p-Tyr (PY99) (1:1000; mouse monoclonal; Santa Cruz Biotechnology #SC-7020, Italy) and anti-actin (1:10000; rabbit polyclonal; Sigma Aldrich #A2103) antibodies in TBST for 1 hour at room temperature (RT) with gentle agitation. After washing, membranes were incubated with secondary antibodies (1:10000 anti-rabbit IgG, HRP-linked or 1:10000 anti-mouse IgG, HRP-linked, depending on the primary antibody isotype; Jackson ImmunoResearch Laboratories Inc. #715-035-150 (Mouse) or #711-035-152 (Rabbit)) for 30–60 minutes at RT with shaking, followed by washing with TBST. If necessary, a specific anti-phosphotyrosine antibody recognizing phosphorylated tyrosine 8 of band 3 was used in place of the nonspecific anti-phosphotyrosine antibody, at a 1:5000 dilution. The anti-

phosphotyrosine 8 antibody was developed in our laboratory with assistance from Proteintech Inc. (Proteintech; antigen name: Li2760-EC1). The mouse monoclonal anti-band 3 antibody was purchased from Sigma Chemical Co. (B9277) and used at a 1:10000 dilution for band 3 staining. Protein visualization was achieved by incubating membranes with a chemiluminescent substrate, followed by imaging using a ChemiDoc Imaging System and Image Lab software (Bio-Rad).[136]

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.0 software (GraphPad, San Diego, CA, USA). For each treatment, two separate experiments with three technical replicates were performed. Two-way analysis-of-variance ANOVA tests with Tukey's correction and the Wilcoxon signed-rank test were used, assuming a p value < 0.05 as statistically significant. We considered * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Results

Microgravity Affects Stemness Gene Expression

Figure 1 illustrates that after 6 hours of culture in the clinostat, Oct-4 expression is markedly upregulated, whereas SOX2 and NANOG levels remain comparable to those observed in control cells maintained under normal gravity conditions. However, prolonged exposure to simulated microgravity (12, 24, and 48 hours) results in a significant downregulation of all three pluripotency markers Oct-4, SOX2, and NANOG whose expression becomes barely detectable over time. [98]

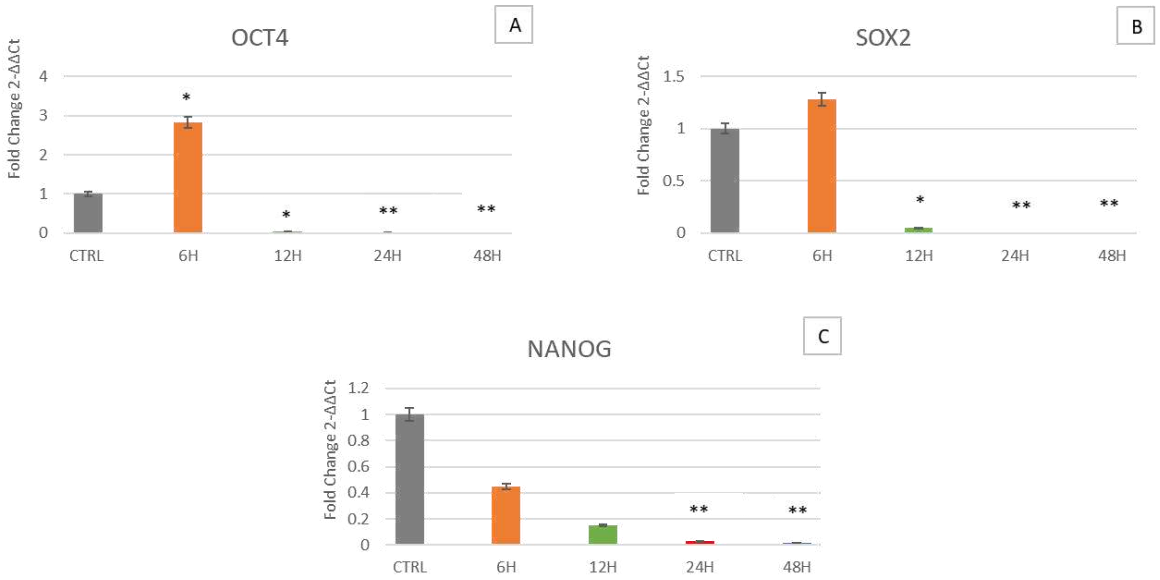


Figure 1. Expression of stemness genes. The expression of the stemness-related genes Oct-4 (**A**), SOX2 (**B**) and NANOG (**C**) was assessed in WJ-MSCs cultured under simulated microgravity (μg) for 6 h-μg (orange bars), 12 h-μg (green bars), 24 h-μg (red bars) or 48 h-μg (blue bars). The mRNA levels for each gene were normalized against glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and were expressed as fold of change ($2^{-\Delta\Delta Ct}$) in mRNA levels observed in controls WJ-MSCs (Ctrl). Controls WJ-MSCs (gray bars) are defined as 1 (mean ± SD; $n = 6$). Data are expressed as mean ± SD referenced to control (* $p \leq 0.05$), (** $p \leq 0.01$). [98]

Simulated Microgravity Affects a Molecular Pattern of Stress Response

Figure 2 shows that in cells cultured for 6 hours in the clinostat, SIRT1 expression is upregulated. After 12 hours, its expression returns to levels comparable to those observed in control cells (Figure 2A). In cells cultured for 24 and 48 hours, SIRT1 expression gradually declines, aligning with control levels. A similar temporal pattern is observed for HSP70 expression (Figure 2C). In contrast, HSP60 (Figure 2B) and NOX4 (Figure 2D) are significantly overexpressed during the initial 6 and 12 hours of microgravity exposure, but their expression progressively diminishes after 24 hours of culture, approaching baseline levels. [98]

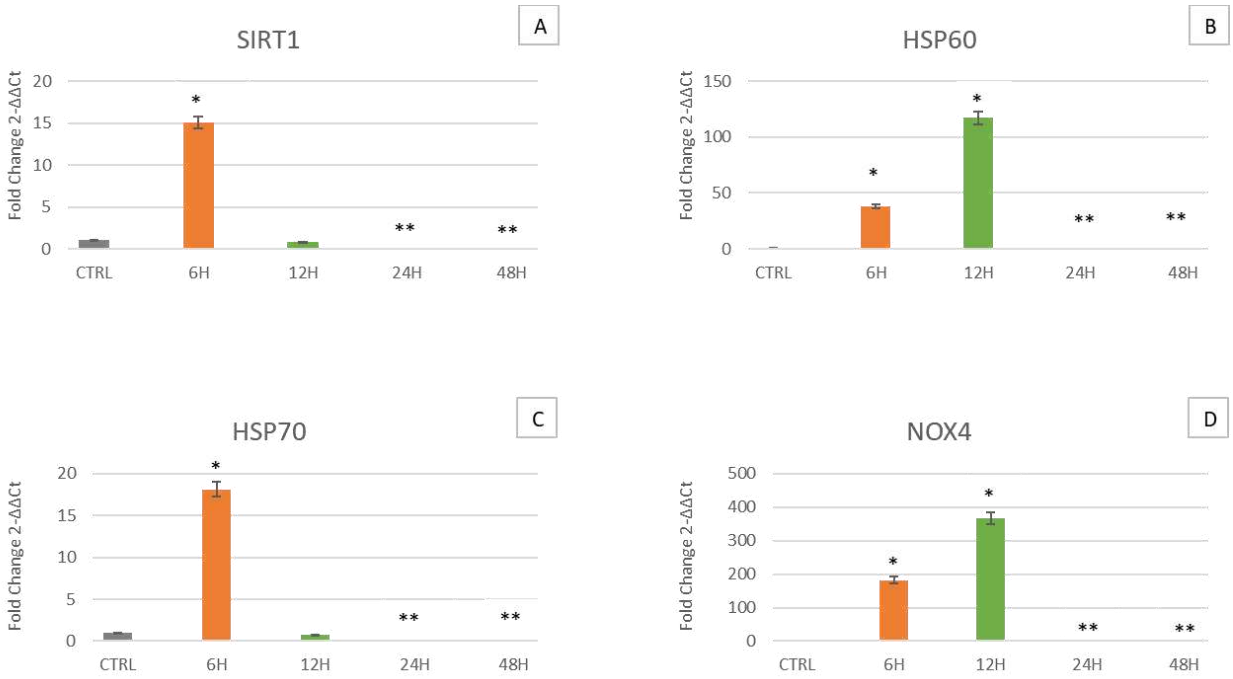


Figure 2. Analysis of sirtuins, heat shock protein and NADPH oxidase. Expression of SIRT1 (**A**), HSP60 (**B**), HSP70 (**C**), and NOX4 (**D**) was assessed in WJ-MSCs cultured under simulated microgravity for microgravity (μg) for 6 h-μg (orange bars), 12 h-μg (green bars), 24 h-μg (red bars) or 48 h-μg (blue bars). The mRNA levels for each gene were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and were expressed as fold of change ($2^{-\Delta\Delta Ct}$) in the mRNA levels observed in controls WJ-MSCs (Ctrl). Controls WJ-MSCs (gray bars) are defined as 1 (mean ± SD; *n* = 6). Data are expressed as mean ± SD referred to control (* $p \leq 0.05$), (** $p \leq 0.01$). [98]

2.3. Simulated Microgravity Activates a Molecular Program of Cell Senescence

Figure 3 demonstrates that after 6 hours of culture in the clinostat, the expression of p16 (Figure 3A), p19 (Figure 3B), p21 (Figure 3C), and p53 (Figure 3D) is upregulated compared to control cells. As the exposure to microgravity continues, the expression levels of p19, p21, and p53 progressively decrease at 12, 24, and 48 hours, eventually reaching levels comparable to controls (grey bars). Interestingly, p16 exhibits a slightly different trend: although it is still overexpressed at 12 hours, its expression decreases after 24 and 48 hours, aligning with the overall pattern of downregulation observed for the other genes. [98]

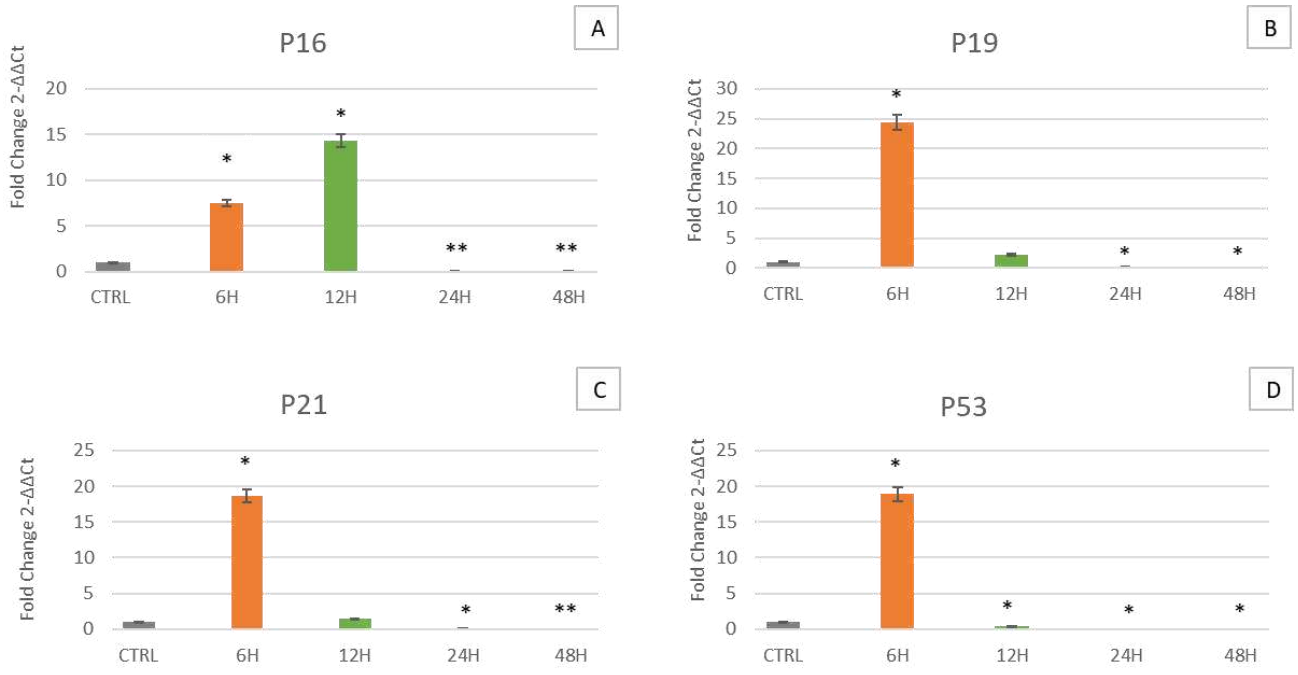


Figure 3. Expression of specific senescence-related markers p16, p19ARF, p21 and p53. Expression of p16 (**A**), p19ARF (**B**), p21 (**C**) and p53 (**D**) was assessed in WJ-MSCs cultured under simulated microgravity (μg) for 6 h-μg (orange bars), 12 h-μg (green bars), 24 h-μg (red bars) or 48 h-μg (blue bars). The mRNA levels for each gene were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and were expressed as fold of change ($2^{-\Delta\Delta Ct}$) in the mRNA levels observed in controls WJ-MSCs (Ctrl). Controls WJ-MSCs (gray bars) are defined as 1 (mean ± SD; *n* = 6). Data are expressed as mean ± SD referred to control (* $p \leq 0.05$), (** $p \leq 0.01$). [98]

2.4. Simulated Microgravity Modulates the Expression of Bax and Bcl2 Apoptotis Related Genes

Figure 4 shows that BAX expression is upregulated in cells cultured for 6 hours in the clinostat compared to control cells. However, with prolonged exposure (12, 24, and 48 hours), its expression progressively declines, returning to levels comparable to those of the controls (Figure 4A). A similar trend is observed for Bcl-2 expression (Figure 4B), which also decreases over time following an initial upregulation. [98]

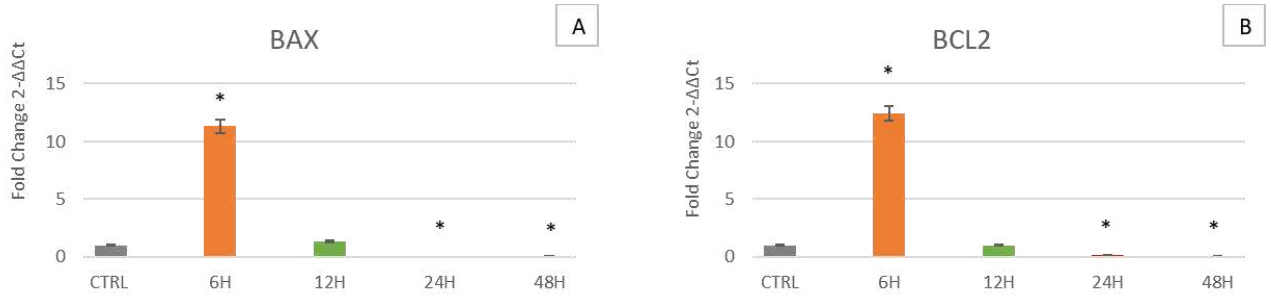


Figure 4. Expression of the apoptotic markers BAX and Bcl-2. Expression of BAX (**A**) and Bcl-2 (**B**) was assessed in WJ-MSCs cultured under simulated microgravity for microgravity (μg) for 6 h-μg (orange bars), 12 h-μg (green bars), 24 h-μg (red bars) or 48 h-μg (blue bars). The mRNA levels for each gene were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and were expressed as fold of change ($2^{-\Delta\Delta Ct}$) in the mRNA levels observed in controls WJ-MSCs (Ctrl). Controls WJ-MSCs (gray bars) are defined as 1 (mean ± SD; *n* = 6). Data are expressed as mean ± SD referred to control (* $p \leq 0.05$). [98]

2.5. Effects of Simulated Microgravity on the Cytoskeleton

Figure 5 indicates that β-Tubulin expression is upregulated in cells cultured for 6 hours in the clinostat compared to control cells. However, after 12, 24, and 48 hours, its expression progressively declines, eventually aligning with control levels (Figure 5B). A similar trend is observed for β-Actin (Figure 5A),

which shows a transient upregulation at 12 hours under microgravity, followed by a gradual decrease at 24 and 48 hours, reaching levels comparable to those of the controls. [98]

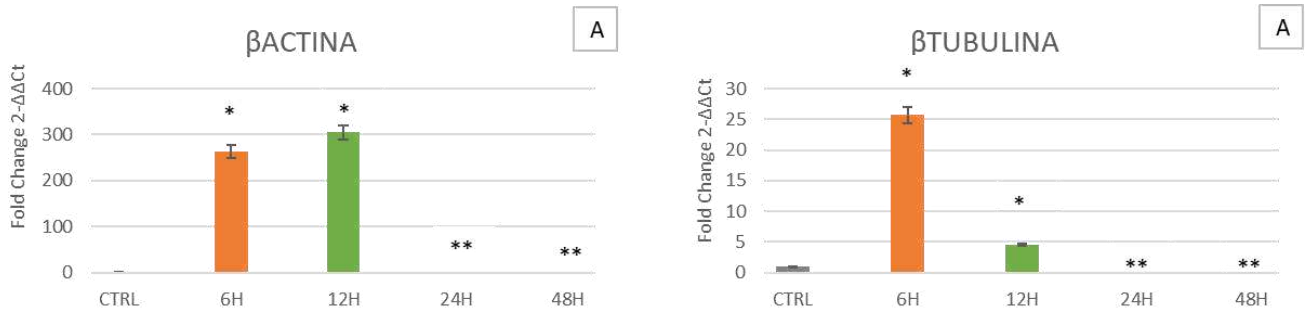


Figure 5. Expression of the principal cytoskeleton markers β-Actin and β-Tubulin. Expression of β-Actin (**A**) and β-Tubulin (**B**) was assessed in WJ-MSCs cultured under simulated microgravity for microgravity (μg) for 6 h-μg (orange bars), 12 h-μg (green bars), 24 h-μg (red bars) or 48 h-μg (blue bars). The mRNA levels for each gene were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and were expressed as fold of change ($2^{-\Delta\Delta Ct}$) in the mRNA levels observed in controls WJ-MSCs (Ctrl). Controls WJ-MSCs (gray bars) are defined as 1 (mean ± SD; $n = 6$). Data are expressed as mean ± SD referred to control (* $p \leq 0.05$), (** $p \leq 0.01$). [98]

2.6. Expression of Cytochrome C under Microgravity

Immunofluorescence images reveal the expression pattern of cytochrome C (Cyt C) in WJ-MSCs cultured under simulated microgravity conditions. After 6 hours of exposure, Cyt C levels appear comparable to those in control cells. However, starting from 12 hours of microgravity exposure, a noticeable increase in Cyt C expression is observed, which persists at later time points when compared to control cells (Ctrl) (Figure 6). [98]

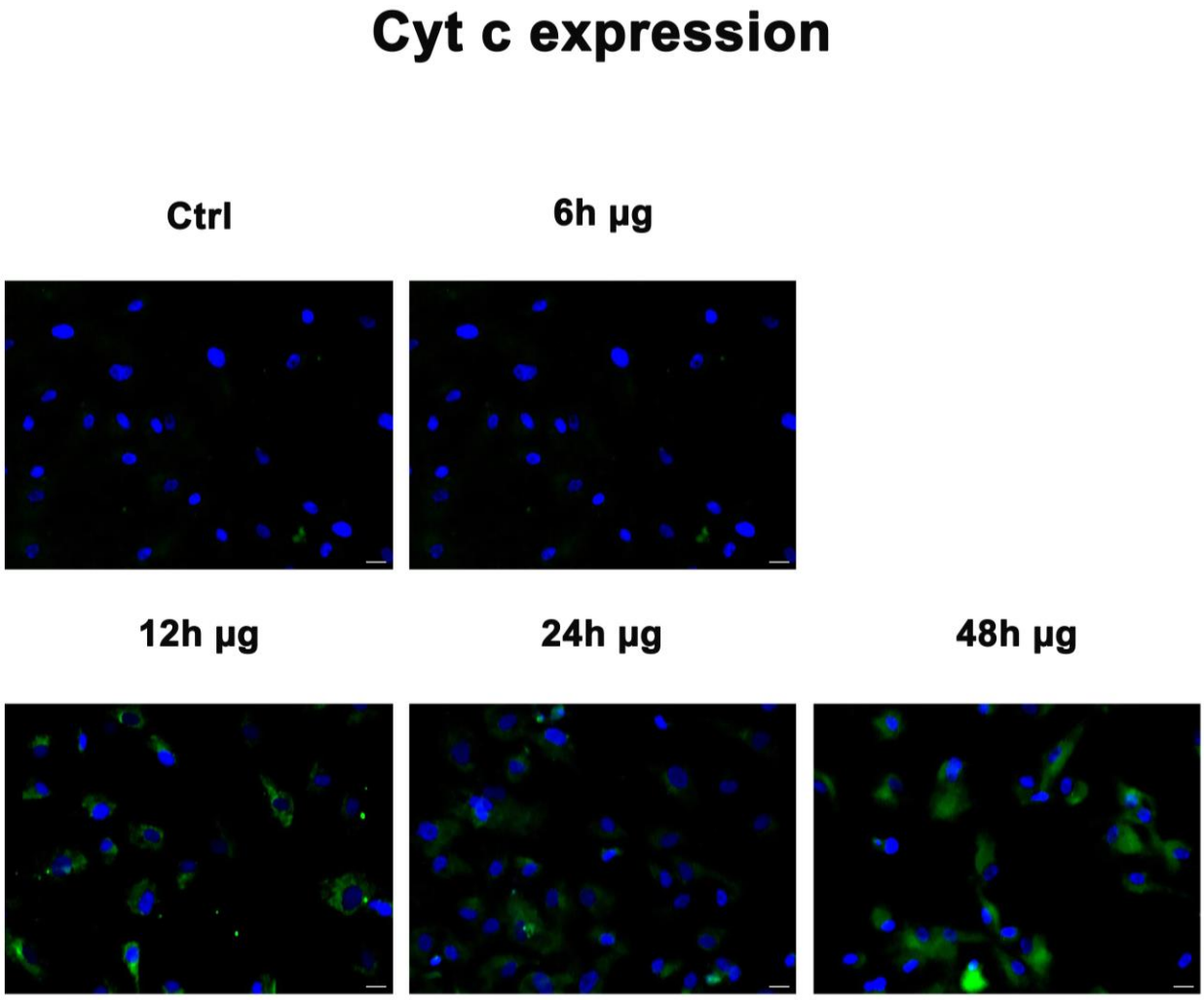


Figure 6. Analysis of Cyt C. Immunofluorescence analysis of Cyt C (green) was assessed in controls WJ-MSCs (Ctrl), and WJ-MSCs incubated from 6 to 48 h under microgravity conditions (µg). The figures are representative of different independent experiments. Nuclei are labelled with 4,6-diamidino-2-phenylindole (DAPI, blue). Magnification 40×. Scale bars: 40 µm. [98]

2.7. Morphological Analysis of WJ-MSCs Exposed to Microgravity

Figure 7 illustrates the morphology of WJ-MSCs following exposure to simulated microgravity. Cells were examined using optical microscopy (Leica, Nussloch, Germany) after 6, 12, 24, and 48 hours under microgravity conditions, as previously described. At all time points, no noticeable morphological differences were observed between cells cultured under microgravity and control (Ctrl) conditions. [98]

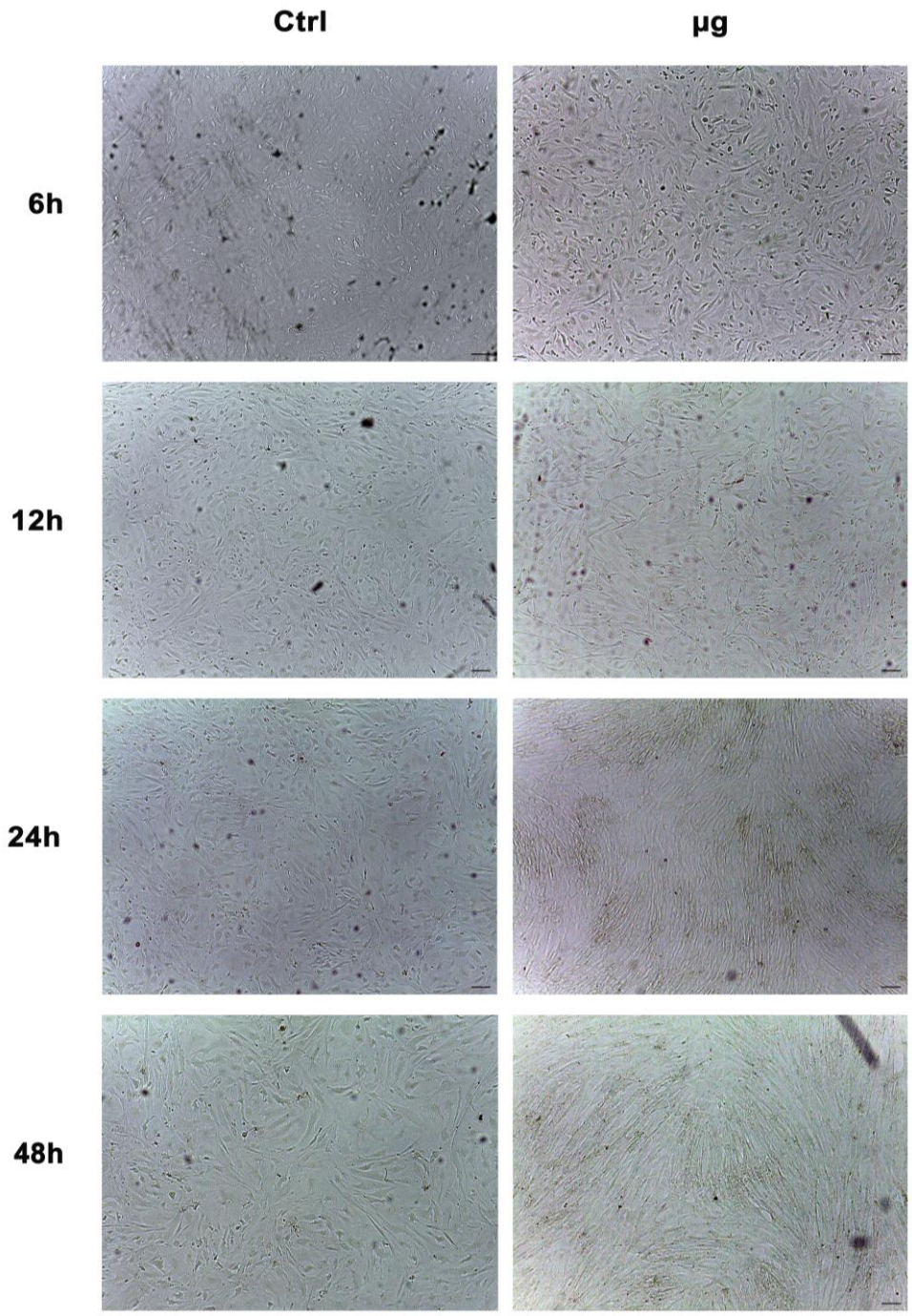


Figure 7. Optical microscope analysis of WJ-MSC morphology after exposure to microgravity (µg). Figure shows morphological changes in cells treated for 6, 12, 24 and 48 h in µg as compared to controls (Ctrl). Scale bar = 100 µm. [98]

2.8. Evaluation of Protein Expression

Figure 8 presents a western blot analysis of cells cultured for 6, 12, 24, and 48 hours under microgravity, with expression levels quantified in arbitrary units. The results confirm the same trend observed in the gene expression analysis. [98]

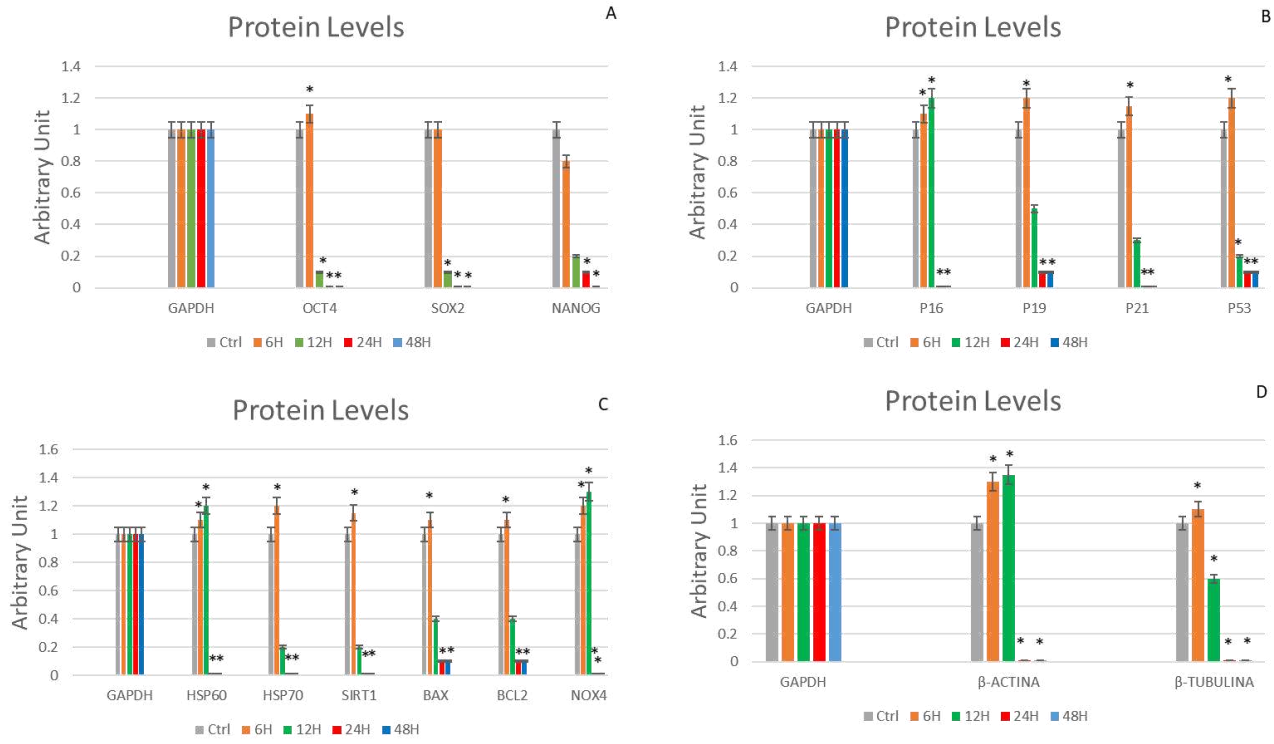


Figure 8. Western blot analysis. Levels of Oct-4, SOX2 and NANOG proteins shown in arbitrary units (**A**). (**B**): levels of p16, p19, p21 and p53 protein shown in arbitrary units. (**C**): levels of Hsp60, Hsp70, Sirt1, Bax, Bcl2 and Nox4 proteins shown in arbitrary units. (**D**): levels of β-Actin and β-Tubulin protein shown in arbitrary units was assessed in WJ-MSCs cultured under simulated microgravity (μg) for 6 h-μg (orange bars), 12 h-μg (green bars), 24 h-μg (red bars) or 48 h-μg (blue bars). The protein levels for each gene were compared against glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). Controls WJ-MSCs (gray bars) are defined as 1 (mean ± SD; $n = 6$). Data are expressed as mean ± SD referenced to control (* $p \leq 0.05$), (** $p \leq 0.01$). [98]

Discussion

Simulated microgravity mimics a space environment, allowing us to unravel cellular behavior and analyze different mechanisms able to face this stressing condition. Here we decided to examine the effect of this condition on WJ-MSCs, a particular type of mesenchymal stem cell, that for their extremely young phenotype exhibit high plasticity and differentiation potential, being more sensitive to any kind of external stimulus. Specifically, WJ-MSCs were cultured for 6, 12, 24 and 48 h under simulated microgravity while control WJ-MSCs were placed in a static bar at 1 g to undergo the same

vibrations as the samples under microgravity conditions. The gene expression analysis of the stemness-related genes demonstrates that simulated microgravity significantly affects the differentiation potential of WJ-MSCs (Figure 1). In particular, after 6 h of clinostat treatment, Oct-4 is initially overexpressed, while SOX2 and NANOG show no significant differences. However, after longer culturing under microgravity, Oct-4, SOX2 and NANOG gene expression is inhibited. The early observed Oct-4 overexpression can drive a metabolic reprogramming of cells, redirecting glucose catabolism to the glycolysis pathway and acting as a tentative mechanism to counteract senescence [137] After 12, 24 and 48 h of clinostat exposure, Oct-4, SOX2 and NANOG were downregulated, indicating that microgravity, acts mainly on Oct-4 in promoting cellular senescence processes [138]. Analysis of the mRNA levels of p16, p19, p21 (WAF1/CIP1) and p53 involved in cell cycle arrest and in cell senescence showed that exposure to µg can, at the beginning, induce the expression of p16, p19, p21 and p53 (**Figure 3**, orange bars). However, after longer periods of exposure to µg (12, 24 and 48 h), a significant overexpression of p16 could be observed while downregulating after 24 and 48 h of culturing under µg, as compared to controls. Meanwhile, after longer periods of exposure to µg (12, 24 and 48 h), a significant downregulation of p19, p21 (WAF1/CIP1) and p53 genes could be observed as compared to controls. A number of regulatory proteins have been suggested to be signals that induce senescence or mediate cell entry into senescence. p16 accumulates as cells undergo increasing numbers of cell divisions and approach senescence [139]. Inactivation of p19Arf accelerates sarcopenia and fat loss, suggesting that its induction suppresses cellular stress that places cells in a state of senescence [140]. p19Arf acts at the nucleolus, where, by binding to other factors, it activates ribosome biogenesis and initiates the p53-dependent processes of senescence and apoptosis[141], [142] . There is a mild senescent cell state which allows cells to resume proliferation after p53 inactivation and a deep senescent state that is irreversible [143]. Therefore, considering our results, we can assume that WJ-MSCs exposed to microgravity undergo a mild senescence state, also partially losing stemness features. Within this context, other authors previously reported that stem cells in the senescent state express modified features, interfering with their stemness and regenerative potential [144]. The impact of individual p53 target genes on the regulation of cellular senescence is highly intricate and context-dependent. In this study, we specifically focused on the roles of key regulators such as p16^INK4a, p19^ARF, p21^CIP1, and p53 itself—a pivotal transcription factor that orchestrates cell cycle arrest and promotes the onset of cellular senescence in response to various stress signals [145], [146]. In addition to identifying p53 as the central mediator of p19^Arf-induced anti-aging responses, this study highlights p21^CIP1 as a crucial downstream effector through which p53 exerts its protective functions. Under stressogenic conditions, aging responses are triggered but are finely modulated by p53, whose action may involve one or multiple downstream target genes depending on the specific tissue context. Moreover, beyond its role in cell cycle regulation and senescence, p53 is also intricately involved in the induction of apoptosis, primarily through the transcriptional activation of

pro-apoptotic proteins such as BAX and the regulation of anti-apoptotic factors like Bcl-2, thus maintaining a critical balance between survival and programmed cell death.[147] Interestingly, our findings reveal that exposure to microgravity induces the expression of both BAX and Bcl-2 genes with opposing roles in the regulation of apoptosis, pro-apoptotic and anti-apoptotic respectively after a short period of culture. However, prolonged exposure leads to their downregulation, suggesting a dynamic and temporally regulated response. This apparent contradiction may indicate the involvement of an upstream regulatory factor that modulates the balance between survival and apoptosis, ultimately contributing to cellular adaptation and survival under microgravity conditions. From a cellular standpoint, mitochondria well established as the primary energy producers also play pivotal roles in various essential processes, including cell cycle progression, metabolic regulation, and the intrinsic apoptotic pathway. Their central role positions them as likely mediators in the cellular response to altered gravitational forces [148]. Interestingly, mitochondrial dysfunction and cellular senescence are both widely recognized as hallmark features of aging. In our study, the observed release of cytochrome c (Figure 6) suggests the activation of mitochondrial-mediated signaling pathways, potentially marking an early step in the metabolic reprogramming associated with stress responses. This finding aligns with the notion that mitochondria not only generate energy but also act as central hubs in the regulation of cell fate. Furthermore, previous studies have demonstrated a strong correlation between high levels of heat shock proteins (HSPs) and increased longevity. Conversely, a decline in HSP expression has been linked to compromised proteostasis, characterized by progressive protein damage, impaired quality control mechanisms, cellular degeneration, and ultimately, cell death [149]. Two of the primary targets of our study are HSP60 and HSP70, which, beyond their well-established roles as molecular chaperones involved in the proper folding, transport, and assembly of newly synthesized polypeptides, also play crucial roles in cellular protection against stress. These heat shock proteins contribute to the maintenance of proteostasis, prevent protein aggregation, and are actively involved in modulating apoptosis, inflammation, and mitochondrial function making them key players in the cellular response to microgravity and aging-related stressors [150], [151], HSP60 and HSP70 not only assist in protein folding and mitochondrial function but also protect cells from a wide range of apoptotic stimuli, including heat shock, tumor necrosis factor (TNF), growth factor withdrawal, oxidative stress, chemotherapeutic agents, and ionizing radiation. Their cytoprotective roles highlight their significance in stress adaptation mechanisms, particularly under conditions such as microgravity where cells are exposed to atypical environmental challenges [152], [153], [154], [155], [156]. Intracellular HSP60 and HSP70 are known to exert multiple cytoprotective effects, including anti-apoptotic and anti-inflammatory functions. Under stress conditions, HSP70 plays a pivotal role in maintaining cellular homeostasis by inhibiting key steps in the apoptotic cascade, notably through the prevention of caspase-3 activation. This mechanism contributes to the preservation of cell viability in response to various internal and external stressors, further underscoring the essential role

of heat shock proteins in the cellular defense machinery [157], [158]. Despite significant recent advances in understanding its cellular functions, the precise anti-apoptotic mechanisms of HSP70 remain a subject of ongoing debate. While several studies have demonstrated its ability to interfere with key apoptotic regulators such as by inhibiting caspase activation, stabilizing mitochondrial membranes, or modulating stress-activated signaling pathways the exact molecular interactions and context-specific effects of HSP70 are still not fully elucidated [157], [159]. In fact, no direct correlation between cytochrome c (Cyt C) release and apoptosis has been consistently reported in the literature. This is because Cyt C can be released from the mitochondria even in viable cells, as suggested by our morphological analysis (Figure 7), which shows no overt signs of cell death despite the presence of Cyt C in the cytoplasm. Furthermore, cells can undergo apoptosis through alternative mechanisms that do not necessarily involve the release of Cyt C, highlighting the complexity of apoptotic pathways and the need for a broader understanding of cellular responses to stress. [160], [161]. Our results align well with the established role of HSP60 and HSP70 in inhibiting apoptosis downstream of cytochrome c (Cyt C) release, but upstream of caspase-3 activation. Specifically, we observed a significant increase in both HSP60 and HSP70 gene expression, concomitant with elevated detection of Cyt C in the cytoplasm (Figure 6), suggesting a protective mechanism that mitigates apoptotic progression. Additionally, the protein SIRT1 has been implicated in the regulation of cellular lifespan and stress resistance, further supporting the hypothesis that cellular longevity mechanisms, including those mediated by heat shock proteins, play a critical role in response to environmental stressors [162], [163], [164]. It has been found that lower circulating levels of SIRT1 in the sera of elderly individuals serve as a distinctive biomarker of cellular senescence. This decline in SIRT1 expression is thought to reflect the diminished ability of cells to respond to stress, contributing to the aging process and the onset of age-related diseases. The correlation between reduced SIRT1 levels and cellular senescence further underscores the potential of SIRT1 as a target for therapeutic strategies aimed at delaying aging and enhancing stress resilience [131]. SIRT1 activity declines rapidly during cellular senescence, with a reduction in NAD+ cofactor levels being considered the primary factor contributing to this decline as individuals age. SIRT1 is ubiquitously expressed in various tissues and plays a crucial role in the progression of numerous age-related diseases, including neurodegeneration, metabolic disorders, and cardiovascular diseases. Notably, activation of SIRT1 has been shown to significantly suppress the expression of p21, a key regulator of cell cycle arrest, thereby mitigating the processes of senescence and promoting cellular longevity. This suggests that SIRT1 could be a promising therapeutic target for delaying aging and preventing age-related cellular dysfunctions [165]. In our study, we demonstrate that SIRT1 is induced early in microgravity (Figure 4), followed by a subsequent downregulation. Notably, SIRT1 activation appears to counteract the expression of the p21 gene, leading to its eventual downregulation (Figure 2), suggesting a potential mechanism for mitigating senescence in response to microgravity. In addition, NOX4 plays a protective role in the vascular system by counteracting inflammatory stress. One study

has shown that NOX4 facilitates beneficial adaptive responses to exercise through the mediation of reactive oxygen species (ROS). However, the downregulation of NOX4 in skeletal muscle during aging and obesity has been linked to the development of insulin resistance and an increase in oxidative stress, further emphasizing its crucial role in maintaining metabolic and cellular homeostasis.[166] In space, the force of gravity is significantly reduced, creating a microgravity (μg) environment. While human cells lack specialized gravity sensors, they are still capable of detecting and responding to μg through mechanotransduction pathways, primarily mediated by the cytoskeleton. The cytoskeletal network acts as a key transducer, allowing cells to sense mechanical changes in their environment and adapt to the altered gravitational forces. This ability to perceive and respond to μg is crucial for understanding how cellular processes, such as growth, differentiation, and stress responses, are modulated under spaceflight conditions [167]. We observed that the cellular response to microgravity (μg) led to a time-dependent modulation of cytoskeletal proteins, with distinct changes in their expression levels at different exposure times. This dynamic regulation highlights the cellular ability to adapt to the altered mechanical environment of microgravity, reflecting the complexity of cytoskeletal remodeling in response to stress. Specifically, β-actin gene expression was significantly upregulated after 6 and 12 hours of exposure, while a marked downregulation was noted after 24 and 48 hours. In contrast, β-tubulin expression showed an initial increase after 6 hours of μg treatment, followed by a subsequent downregulation at 12, 24, and 48 hours. These findings suggest that microgravity initially promotes cytoskeletal adaptation, potentially facilitating the cell's ability to cope with altered mechanical forces. However, this adaptation appears to diminish over time, leading to a weakening of the cellular scaffold and possibly compromising cell integrity at later stages of exposure [168]. Overall, all the genes analyzed in this study exhibited a similar temporal expression pattern, showing upregulation in cells exposed to microgravity for 6 hours, followed by a subsequent downregulation after longer periods of exposure. Based on these findings, WJ-MSCs exposed to microgravity appear to initiate a rapid adaptive stress response within the first 6 hours, which results in the increased expression of genes associated with stemness, stress response, and apoptosis/senescence. However, following this initial adaptive phase, cells undergo more pronounced cellular adaptation, characterized by the downregulation of these genes and the eventual onset of senescence. Notably, the observed release of cytochrome c (Cyt C) and metabolic cellular adaptation seem to act as critical triggers, steering the cells toward senescence and highlighting the complex interplay between stress responses and cellular fate decisions under microgravity conditions. [98]

Conclusions

In this study, we conducted a comprehensive analysis of the behavior and functional characteristics of Mesenchymal Stem Cells derived from Wharton’s Jelly (WJ-MSCs) when exposed to simulated microgravity conditions, with the aim of understanding how this unique environment influences their

morphology, proliferation, differentiation potential, and gene expression profiles. Our findings reveal an early and robust cellular response to microgravity-induced stress, which is subsequently followed by the establishment of an adaptive senescence state. This adaptive phase appears to be stabilized through a complex interplay of various molecular and cellular factors, including alterations in gene expression and cytoskeletal dynamics. Specifically, our data demonstrate that microgravity not only influences the expression of key genes involved in differentiation, stress response, and senescence pathways, but also triggers significant cytoskeletal rearrangements, potentially affecting cellular morphology, mechanotransduction, and overall functionality. These structural and genetic changes suggest a multifaceted cellular adaptation process aimed at maintaining homeostasis in an altered gravitational environment. While these results offer valuable preliminary insights, further in-depth studies are required to elucidate the precise molecular mechanisms and signaling pathways governing the cellular response to microgravity. Such investigations could help identify potential targets for mitigating adverse effects on stem cell functionality during prolonged space missions. Nevertheless, our current findings provide a foundational understanding of how microgravity impacts stem cell fate and tissue regenerative potential. This knowledge not only contributes to the broader field of space biology but also paves the way for developing future protective strategies to preserve stem cell viability and enhance tissue regeneration in microgravity environments. Ultimately, these insights hold significant implications for advancing regenerative medicine applications in both space exploration and terrestrial health setting.

References

[1] M. Sharpe, G. Leoni, and J. Hyllner, “Stem Cells,” in Comprehensive Toxicology: Third Edition, 2017.

[2] G. Kolios e Y. Moodley, «Introduction to Stem Cells and Regenerative Medicine», Respiration, vol. 85, fasc. 1, pp. 3–10, dic. 2012, doi: 10.1159/000345615.

[3] A. Vats, R. C. Bielby, N. S. Tolley, R. Nerem, e J. M. Polak, «Stem cells», Lancet, vol. 366, fasc. 9485, pp. 592–602, ago. 2005, doi: 10.1016/S0140-6736(05)66879-1.

[4] S. He, D. Nakada, e S. J. Morrison, «Mechanisms of stem cell self-renewal», Annu Rev Cell Dev Biol, vol. 25, pp. 377–406, 2009, doi: 10.1146/annurev.cellbio.042308.113248.

[5] M. Mirotsou, T. M. Jayawardena, J. Schmeckpeper, M. Gnecchi, e V. J. Dzau, «Paracrine mechanisms of stem cell reparative and regenerative actions in the heart», J Mol Cell Cardiol, vol. 50, fasc. 2, pp. 280–289, feb. 2011, doi: 10.1016/j.yjmcc.2010.08.005.

[6] F. Balzano et al., «MiR200 and miR302: Two Big Families Influencing Stem Cell Behavior», Molecules, vol. 23, fasc. 2, p. 282, gen. 2018, doi: 10.3390/molecules23020282.

[7] R. S et al., «Regenerative treatment using a radioelectric asymmetric conveyor as a novel tool in antiaging medicine: an in vitro beta-galactosidase study», Clinical interventions in aging, vol. 7, 2012, doi: 10.2147/CIA.S33312.

[8] M. Maioli et al., «REAC technology and hyaluron synthase 2, an interesting network to slow down stem cell senescence», Sci Rep, vol. 6, p. 28682, giu. 2016, doi: 10.1038/srep28682.

[9] D. Ilic e J. M. Polak, «Stem cells in regenerative medicine: introduction», Br Med Bull, vol. 98, pp. 117–126, 2011, doi: 10.1093/bmb/ldr012.
[10] M. J. Evans e M. H. Kaufman, «Establishment in culture of pluripotential cells from mouse embryos», Nature, vol. 292, fasc. 5819, pp. 154–156, lug. 1981, doi: 10.1038/292154a0.
[11] S. Yao et al., «Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions», Proc Natl Acad Sci U S A, vol. 103, fasc. 18, pp. 6907–6912, mag. 2006, doi: 10.1073/pnas.0602280103.
[12] A. Bongso, «Blastocyst culture for deriving human embryonic stem cells», Methods Mol Biol, vol. 331, pp. 13–22, 2006, doi: 10.1385/1-59745-046-4:13.
[13] Z. Wang, E. Oron, B. Nelson, S. Razis, e N. Ivanova, «Distinct lineage specification roles for NANOG, OCT4, and SOX2 in human embryonic stem cells», Cell Stem Cell, vol. 10, fasc. 4, pp. 440–454, apr. 2012, doi: 10.1016/j.stem.2012.02.016.
[14] J. Nichols et al., «Formation of Pluripotent Stem Cells in the Mammalian Embryo Depends on the POU Transcription Factor Oct4», Cell, vol. 95, fasc. 3, pp. 379–391, ott. 1998, doi: 10.1016/S0092-8674(00)81769-9.
[15] N. Ivanova et al., «Dissecting self-renewal in stem cells with RNA interference», Nature, vol. 442, fasc. 7102, pp. 533–538, 2006, doi: 10.1038/nature04915.
[16] C. Chazaud, Y. Yamanaka, T. Pawson, e J. Rossant, «Early Lineage Segregation between Epiblast and Primitive Endoderm in Mouse Blastocysts through the Grb2-MAPK Pathway», Developmental Cell, vol. 10, fasc. 5, pp. 615–624, mag. 2006, doi: 10.1016/j.devcel.2006.02.020.
[17] S. Masui et al., «Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells», Nature Cell Biology, vol. 9, fasc. 6, pp. 625–635, 2007, doi: 10.1038/ncb1589.
[18] L. A. Boyer et al., «Core transcriptional regulatory circuitry in human embryonic stem cells», Cell, vol. 122, fasc. 6, pp. 947–956, 2005, doi: 10.1016/j.cell.2005.08.020.
[19] X. Chen et al., «Integration of External Signaling Pathways with the Core Transcriptional Network in Embryonic Stem Cells», Cell, vol. 133, fasc. 6, pp. 1106–1117, 2008, doi: 10.1016/j.cell.2008.04.043.
[20] D. G. Zacharias, T. J. Nelson, P. S. Mueller, e C. C. Hook, «The Science and Ethics of Induced Pluripotency: What Will Become of Embryonic Stem Cells?», Mayo Clin Proc, vol. 86, fasc. 7, pp. 634–640, lug. 2011, doi: 10.4065/mcp.2011.0054.
[21] E. Hiyama e K. Hiyama, «Telomere and telomerase in stem cells», Br J Cancer, vol. 96, fasc. 7, pp. 1020–1024, apr. 2007, doi: 10.1038/sj.bjc.6603671.
[22] Van Deursen e J. M, «The role of senescent cells in ageing», Nature, vol. 509, fasc. 7501, pp. 439–446, mag. 2014, doi: 10.1038/nature13193.
[23] P. A. Zuk et al., «Multilineage cells from human adipose tissue: implications for cell-based therapies», Tissue Eng, vol. 7, fasc. 2, pp. 211–228, apr. 2001, doi: 10.1089/107632701300062859.
[24] A. Malgieri, E. Kantzari, M. P. Patrizi, e S. Gambardella, «Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art», Int J Clin Exp Med, vol. 3, fasc. 4, pp. 248–269, set. 2010.
[25] A. J. Wagers e I. L. Weissman, «Plasticity of adult stem cells», Cell, vol. 116, fasc. 5, pp. 639–648, mar. 2004, doi: 10.1016/s0092-8674(04)00208-9.
[26] R. S. Mahla, «Stem Cells Applications in Regenerative Medicine and Disease Therapeutics», Int J Cell Biol, vol. 2016, p. 6940283, 2016, doi: 10.1155/2016/6940283.
[27] R. Thambyrajah e A. Bigas, «Notch Signaling in HSC Emergence: When, Why and How», Cells, vol. 11, fasc. 3, p. 358, gen. 2022, doi: 10.3390/cells11030358.
[28] G. A. Challen, N. Boles, K. K.-Y. Lin, e M. A. Goodell, «Mouse hematopoietic stem cell identification and analysis», Cytometry A, vol. 75, fasc. 1, pp. 14–24, gen. 2009, doi: 10.1002/cyto.a.20674.
[29] R. Calloni, E. A. A. Cordero, J. A. P. Henriques, e D. Bonatto, «Reviewing and Updating the Major Molecular Markers for Stem Cells», Stem Cells Dev, vol. 22, fasc. 9, pp. 1455–1476, mag. 2013, doi: 10.1089/scd.2012.0637.
[30] M. F. Pittenger et al., «Multilineage potential of adult human mesenchymal stem cells», Science, vol. 284, fasc. 5411, pp. 143–147, apr. 1999, doi: 10.1126/science.284.5411.143.

[31] A. Arthur e S. Gronthos, «Clinical Application of Bone Marrow Mesenchymal Stem/Stromal Cells to Repair Skeletal Tissue», Int J Mol Sci, vol. 21, fasc. 24, p. 9759, dic. 2020, doi: 10.3390/ijms21249759.
[32] S. Dadfar et al., «The Role of Mesenchymal Stem Cells in Modulating Adaptive Immune Responses in Multiple Sclerosis», Cells, vol. 13, fasc. 18, Art. fasc. 18, gen. 2024, doi: 10.3390/cells13181556.
[33] M. Di Nicola et al., «Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli», Blood, vol. 99, fasc. 10, pp. 3838–3843, mag. 2002, doi: 10.1182/blood.v99.10.3838.
[34] A. I. Caplan, «Mesenchymal Stem Cells: Time to Change the Name!», Stem Cells Transl Med, vol. 6, fasc. 6, pp. 1445–1451, giu. 2017, doi: 10.1002/sctm.17-0051.
[35] T. Yi e S. U. Song, «Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications», Arch Pharm Res, vol. 35, fasc. 2, pp. 213–221, feb. 2012, doi: 10.1007/s12272-012-0202-z.
[36] S. Maxson, E. A. Lopez, D. Yoo, A. Danilkovitch-Miagkova, e M. A. LeRoux, «Concise Review: Role of Mesenchymal Stem Cells in Wound Repair», Stem Cells Transl Med, vol. 1, fasc. 2, pp. 142–149, feb. 2012, doi: 10.5966/sctm.2011-0018.
[37] V. Karantalis e J. M. Hare, «Use of Mesenchymal Stem Cells for Therapy of Cardiac Disease», Circ Res, vol. 116, fasc. 8, pp. 1413–1430, apr. 2015, doi: 10.1161/CIRCRESAHA.116.303614.
[38] P. Hernigou, A. Poignard, F. Beaujean, e H. Rouard, «Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells», J Bone Joint Surg Am, vol. 87, fasc. 7, pp. 1430–1437, lug. 2005, doi: 10.2106/JBJS.D.02215.
[39] H. Le, W. Xu, X. Zhuang, F. Chang, Y. Wang, e J. Ding, «Mesenchymal stem cells for cartilage regeneration», J Tissue Eng, vol. 11, p. 2041731420943839, ago. 2020, doi: 10.1177/2041731420943839.
[40] Y. Guo, Y. Yu, S. Hu, Y. Chen, e Z. Shen, «The therapeutic potential of mesenchymal stem cells for cardiovascular diseases», Cell Death Dis, vol. 11, fasc. 5, p. 349, mag. 2020, doi: 10.1038/s41419-020-2542-9.
[41] I. Arutyunyan, A. Elchaninov, A. Makarov, e T. Fatkhudinov, «Umbilical Cord as Prospective Source for Mesenchymal Stem Cell-Based Therapy», Stem Cells Int, vol. 2016, p. 6901286, 2016, doi: 10.1155/2016/6901286.
[42] E. Di Naro, F. Ghezzi, L. Raio, M. Franchi, e V. D'Addario, «Umbilical cord morphology and pregnancy outcome», Eur J Obstet Gynecol Reprod Biol, vol. 96, fasc. 2, pp. 150–157, giu. 2001, doi: 10.1016/s0301-2115(00)00470-x.
[43] H.-S. Wang et al., «Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord», Stem Cells, vol. 22, fasc. 7, pp. 1330–1337, 2004, doi: 10.1634/stemcells.2004-0013.
[44] D.-W. Kim, M. Staples, K. Shinozuka, P. Pantcheva, S.-D. Kang, e C. V. Borlongan, «Wharton's Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic Potential for Clinical Applications», International Journal of Molecular Sciences, vol. 14, fasc. 6, Art. fasc. 6, giu. 2013, doi: 10.3390/ijms140611692.
[45] K. Takechi, Y. Kuwabara, e M. Mizuno, «Ultrastructural and immunohistochemical studies of Wharton's jelly umbilical cord cells», Placenta, vol. 14, fasc. 2, pp. 235–245, mar. 1993, doi: 10.1016/S0143-4004(05)80264-4.
[46] A. Stolzing, E. Jones, D. McGonagle, e A. Scutt, «Age-related changes in human bone marrow-derived mesenchymal stem cells: Consequences for cell therapies», Mechanisms of Ageing and Development, vol. 129, fasc. 3, pp. 163–173, mar. 2008, doi: 10.1016/j.mad.2007.12.002.
[47] F. V. Paladino, J. S. Peixoto-Cruz, C. Santacruz-Perez, e A. C. Goldberg, «Comparison between isolation protocols highlights intrinsic variability of human umbilical cord mesenchymal cells», Cell Tissue Bank, vol. 17, fasc. 1, pp. 123–136, mar. 2016, doi: 10.1007/s10561-015-9525-6.
[48] I. Kalaszczynska e K. Ferdyn, «Wharton's jelly derived mesenchymal stem cells: future of regenerative medicine? Recent findings and clinical significance», Biomed Res Int, vol. 2015, p. 430847, 2015, doi: 10.1155/2015/430847.

[49] H. Khan, P. Mafi, R. Mafi, e W. Khan, «The Effects of Ageing on Differentiation and Characterisation of Human Mesenchymal Stem Cells», Current Stem Cell Research & Therapy, vol. 13, fasc. 5, pp. 378–383, lug. 2018, doi: 10.2174/1574888X11666160429122527.
[50] V. Sabapathy, B. Sundaram, S. V M, P. Mankuzhy, e S. Kumar, «Human Wharton's Jelly Mesenchymal Stem Cells plasticity augments scar-free skin wound healing with hair growth», PLoS One, vol. 9, fasc. 4, p. e93726, 2014, doi: 10.1371/journal.pone.0093726.
[51] K. Le Blanc, C. Tammik, K. Rosendahl, E. Zetterberg, e O. Ringdén, «HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells», Exp Hematol, vol. 31, fasc. 10, pp. 890–896, ott. 2003, doi: 10.1016/s0301-472x(03)00110-3.
[52] M. Wang et al., «The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro», Immunology, vol. 126, fasc. 2, pp. 220–232, feb. 2009, doi: 10.1111/j.1365-2567.2008.02891.x.
[53] M. L. Weiss et al., «Immune properties of human umbilical cord Wharton's jelly-derived cells», Stem Cells, vol. 26, fasc. 11, pp. 2865–2874, nov. 2008, doi: 10.1634/stemcells.2007-1028.
[54] M. A. Baxter, R. F. Wynn, S. N. Jowitt, J. E. Wraith, L. J. Fairbairn, e I. Bellantuono, «Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion», Stem Cells, vol. 22, fasc. 5, pp. 675–682, 2004, doi: 10.1634/stemcells.22-5-675.
[55] R. Izadpanah et al., «Long-term in vitro expansion alters the biology of adult mesenchymal stem cells», Cancer Res, vol. 68, fasc. 11, pp. 4229–4238, giu. 2008, doi: 10.1158/0008-5472.CAN-07-5272.
[56] C. H. Mun, M.-I. Kang, Y. D. Shin, Y. Kim, e Y.-B. Park, «The Expression of Immunomodulation-Related Cytokines and Genes of Adipose- and Bone Marrow-Derived Human Mesenchymal Stromal Cells from Early to Late Passages», Tissue Eng Regen Med, vol. 15, fasc. 6, pp. 771–779, dic. 2018, doi: 10.1007/s13770-018-0147-5.
[57] Y.-H. K. Yang, C. R. Ogando, C. Wang See, T.-Y. Chang, e G. A. Barabino, «Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro», Stem Cell Res Ther, vol. 9, fasc. 1, p. 131, mag. 2018, doi: 10.1186/s13287-018-0876-3.
[58] P. Fv, S. Lr, P. Ca, e G. Ac, «Intrinsic Variability Present in Wharton's Jelly Mesenchymal Stem Cells and T Cell Responses May Impact Cell Therapy», Stem cells international, vol. 2017, 2017, doi: 10.1155/2017/8492797.
[59] C. R, D. D, W. M, S. B, e T. D, «Expression of early transcription factors Oct-4, Sox-2 and Nanog by porcine umbilical cord (PUC) matrix cells», Reproductive biology and endocrinology : RB&E, vol. 4, giu. 2006, doi: 10.1186/1477-7827-4-8.
[60] M.-R. E, S. D, L. F, G. J, N. C, e W. Sm, «5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies», Vox sanguinis, vol. 95, fasc. 2, ago. 2008, doi: 10.1111/j.1423-0410.2008.01076.x.
[61] N. Beeravolu et al., «Isolation and Characterization of Mesenchymal Stromal Cells from Human Umbilical Cord and Fetal Placenta», Journal of Visualized Experiments : JoVE, fasc. 122, 2017, doi: 10.3791/55224.
[62] M. Xu et al., «The immunologic and hematopoietic profiles of mesenchymal stem cells derived from different sections of human umbilical cord», Acta Biochim Biophys Sin (Shanghai), vol. 46, fasc. 12, pp. 1056–1065, dic. 2014, doi: 10.1093/abbs/gmu100.
[63] C. Zhou et al., «Immunomodulatory effect of human umbilical cord Wharton's jelly-derived mesenchymal stem cells on lymphocytes», Cell Immunol, vol. 272, fasc. 1, pp. 33–38, 2011, doi: 10.1016/j.cellimm.2011.09.010.
[64] E. Karaöz, P. Çetinalp Demircan, G. Erman, E. Güngörürler, e A. Eker Sarıboyacı, «Comparative Analyses of Immunosuppressive Characteristics of Bone-Marrow, Wharton's Jelly, and Adipose Tissue-Derived Human Mesenchymal Stem Cells», Turk J Haematol, vol. 34, fasc. 3, pp. 213–225, ago. 2017, doi: 10.4274/tjh.2016.0171.
[65] Y. Deng et al., «Umbilical cord-derived mesenchymal stem cells instruct dendritic cells to acquire tolerogenic phenotypes through the IL-6-mediated upregulation of SOCS1», Stem Cells Dev, vol. 23, fasc. 17, pp. 2080–2092, set. 2014, doi: 10.1089/scd.2013.0559.
[66] R. Donders et al., «Human Wharton's Jelly-Derived Stem Cells Display a Distinct Immunomodulatory and Proregenerative Transcriptional Signature Compared to Bone Marrow-

Derived Stem Cells», Stem Cells Dev, vol. 27, fasc. 2, pp. 65–84, gen. 2018, doi: 10.1089/scd.2017.0029.
[67] H. Y et al., «Wharton's jelly mesenchymal stem cells differentiate into retinal progenitor cells», Neural regeneration research, vol. 8, fasc. 19, mag. 2013, doi: 10.3969/j.issn.1673-5374.2013.19.006.
[68] W. Briegleb, «Some qualitative and quantitative aspects of the fast-rotating clinostat as a research tool», ASGSB Bull, vol. 5, fasc. 2, pp. 23–30, ott. 1992.
[69] T. Hoson, S. Kamisaka, Y. Masuda, M. Yamashita, e B. Buchen, «Evaluation of the three-dimensional clinostat as a simulator of weightlessness», Planta, vol. 203 Suppl, pp. S187-197, 1997, doi: 10.1007/pl00008108.
[70] J. Z. Kiss, R. E. Edelmann, e P. C. Wood, «Gravitropism of hypocotyls of wild-type and starch-deficient Arabidopsis seedlings in spaceflight studies», Planta, vol. 209, fasc. 1, pp. 96–103, lug. 1999, doi: 10.1007/s004250050610.
[71] «Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology - PubMed». Consultato: 3 febbraio 2025. [Online]. Disponibile su: https://pubmed.ncbi.nlm.nih.gov/23252378/
[72] J. J. W. A. van Loon, «Some history and use of the random positioning machine, RPM, in gravity related research», Advances in Space Research, vol. 39, fasc. 7, pp. 1161–1165, gen. 2007, doi: 10.1016/j.asr.2007.02.016.
[73] C. A. Nickerson, C. M. Ott, S. J. Mister, B. J. Morrow, L. Burns-Keliher, e D. L. Pierson, «Microgravity as a novel environmental signal affecting Salmonella enterica serovar Typhimurium virulence», Infect Immun, vol. 68, fasc. 6, pp. 3147–3152, giu. 2000, doi: 10.1128/IAI.68.6.3147-3152.2000.
[74] B. M. Uva et al., «Clinorotation-induced weightlessness influences the cytoskeleton of glial cells in culture», Brain Res, vol. 934, fasc. 2, pp. 132–139, mag. 2002, doi: 10.1016/s0006-8993(02)02415-0.
[75] L. Xue, Y. Li, e J. Chen, «Duration of simulated microgravity affects the differentiation of mesenchymal stem cells», Mol Med Rep, vol. 15, fasc. 5, pp. 3011–3018, mag. 2017, doi: 10.3892/mmr.2017.6357.
[76] X. Lin et al., «The Impact of Spaceflight and Simulated Microgravity on Cell Adhesion», Int J Mol Sci, vol. 21, fasc. 9, p. 3031, apr. 2020, doi: 10.3390/ijms21093031.
[77] D. Grimm et al., «The effects of microgravity on differentiation and cell growth in stem cells and cancer stem cells», Stem Cells Translational Medicine, vol. 9, fasc. 8, pp. 882–894, ago. 2020, doi: 10.1002/sctm.20-0084.
[78] «The International Space Station as a microgravity research platform | Request PDF», ResearchGate, ott. 2024, doi: 10.1016/S0094-5765(02)00003-6.
[79] W. Mao et al., «Microgravity Inhibits Cell Proliferation and Promotes Senescence and Apoptosis in Embryonic Stem Cells», Space: Science & Technology, vol. 4, p. 0104, apr. 2024, doi: 10.34133/space.0104.
[80] H. P. Nguyen, P. H. Tran, K.-S. Kim, e S.-G. Yang, «The effects of real and simulated microgravity on cellular mitochondrial function», npj Microgravity, vol. 7, fasc. 1, pp. 1–11, nov. 2021, doi: 10.1038/s41526-021-00171-7.
[81] M. Anil-Inevi, E. Yilmaz, O. Sarigil, H. C. Tekin, e E. Ozcivici, «Single Cell Densitometry and Weightlessness Culture of Mesenchymal Stem Cells Using Magnetic Levitation», in Stem Cell Nanotechnology: Methods and Protocols, K. Turksen, A c. di, New York, NY: Springer US, 2020, pp. 15–25. doi: 10.1007/7651_2019_231.
[82] S. S. Raza et al., «Neuroprotective effect of naringenin is mediated through suppression of NF-κB signaling pathway in experimental stroke», Neuroscience, vol. 230, pp. 157–171, gen. 2013, doi: 10.1016/j.neuroscience.2012.10.041.
[83] H. Tsutsui, S. Kinugawa, e S. Matsushima, «Oxidative stress and heart failure», Am J Physiol Heart Circ Physiol, vol. 301, fasc. 6, pp. H2181-2190, dic. 2011, doi: 10.1152/ajpheart.00554.2011.
[84] T. P. Stein e M. J. Leskiw, «Oxidant damage during and after spaceflight», American Journal of Physiology-Endocrinology and Metabolism, vol. 278, fasc. 3, pp. E375–E382, mar. 2000, doi: 10.1152/ajpendo.2000.278.3.E375.

[85] D. Q. Tan e T. Suda, «Reactive Oxygen Species and Mitochondrial Homeostasis as Regulators of Stem Cell Fate and Function», Antioxid Redox Signal, vol. 29, fasc. 2, pp. 149–168, lug. 2018, doi: 10.1089/ars.2017.7273.
[86] C. C. Winterbourn, «Biological Production, Detection, and Fate of Hydrogen Peroxide», Antioxid Redox Signal, vol. 29, fasc. 6, pp. 541–551, ago. 2018, doi: 10.1089/ars.2017.7425.
[87] B. R. Stockwell e X. Jiang, «The Chemistry and Biology of Ferroptosis», Cell Chem Biol, vol. 27, fasc. 4, pp. 365–375, apr. 2020, doi: 10.1016/j.chembiol.2020.03.013.
[88] J. Zheng e M. Conrad, «The Metabolic Underpinnings of Ferroptosis», Cell Metab, vol. 32, fasc. 6, pp. 920–937, dic. 2020, doi: 10.1016/j.cmet.2020.10.011.
[89] R. Liang e S. Ghaffari, «Stem Cells Seen Through the FOXO Lens: An Evolving Paradigm», Curr Top Dev Biol, vol. 127, pp. 23–47, 2018, doi: 10.1016/bs.ctdb.2017.11.006.
[90] S. Cruciani et al., «Melatonin finely tunes proliferation and senescence in hematopoietic stem cells», Eur J Cell Biol, vol. 101, fasc. 3, p. 151251, 2022, doi: 10.1016/j.ejcb.2022.151251.
[91] K. M. Holmström e T. Finkel, «Cellular mechanisms and physiological consequences of redox-dependent signalling», Nat Rev Mol Cell Biol, vol. 15, fasc. 6, pp. 411–421, giu. 2014, doi: 10.1038/nrm3801.
[92] R. Raynes, J. Brunquell, e S. D. Westerheide, «Stress Inducibility of SIRT1 and Its Role in Cytoprotection and Cancer», Genes Cancer, vol. 4, fasc. 3–4, pp. 172–182, mar. 2013, doi: 10.1177/1947601913484497.
[93] S. Patel, «The effects of microgravity and space radiation on cardiovascular health: From low-Earth orbit and beyond», IJC Heart & Vasculature, vol. 30, p. 100595, ott. 2020, doi: 10.1016/j.ijcha.2020.100595.
[94] M. Moreno-Villanueva, M. Wong, T. Lu, Y. Zhang, e H. Wu, «Interplay of space radiation and microgravity in DNA damage and DNA damage response», npj Microgravity, vol. 3, fasc. 1, pp. 1–8, mag. 2017, doi: 10.1038/s41526-017-0019-7.
[95] T. Najrana e J. Sanchez-Esteban, «Mechanotransduction as an Adaptation to Gravity», Front Pediatr, vol. 4, p. 140, dic. 2016, doi: 10.3389/fped.2016.00140.
[96] A. Acharya et al., «Microgravity-induced stress mechanisms in human stem cell-derived cardiomyocytes», iScience, vol. 25, fasc. 7, p. 104577, giu. 2022, doi: 10.1016/j.isci.2022.104577.
[97] P. Huang, B. T. Piatkowski, Y. Cherukuri, Y. W. Asmann, e A. C. Zubair, «Impact of spaceflight on gene expression in cultured human mesenchymal stem/stromal cells», PLOS ONE, vol. 20, fasc. 3, p. e0315285, mar. 2025, doi: 10.1371/journal.pone.0315285.
[98] R. Pala et al., «Mesenchymal Stem Cell Behavior under Microgravity: From Stress Response to a Premature Senescence», Int J Mol Sci, vol. 24, fasc. 9, p. 7753, apr. 2023, doi: 10.3390/ijms24097753.
[99] M. C. Haigis e L. P. Guarente, «Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction», Genes Dev, vol. 20, fasc. 21, pp. 2913–2921, nov. 2006, doi: 10.1101/gad.1467506.
[100] J. Landry, J. T. Slama, e R. Sternglanz, «Role of NAD(+) in the deacetylase activity of the SIR2-like proteins», Biochem Biophys Res Commun, vol. 278, fasc. 3, pp. 685–690, nov. 2000, doi: 10.1006/bbrc.2000.3854.
[101] M. Kaeberlein, M. McVey, e L. Guarente, «Using yeast to discover the fountain of youth», Sci Aging Knowledge Environ, vol. 2001, fasc. 1, p. pe1, ott. 2001, doi: 10.1126/sageke.2001.1.pe1.
[102] H. A. Tissenbaum e L. Guarente, «Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans», Nature, vol. 410, fasc. 6825, pp. 227–230, mar. 2001, doi: 10.1038/35065638.
[103] M. Viswanathan e L. Guarente, «Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes», Nature, vol. 477, fasc. 7365, pp. E1-2, set. 2011, doi: 10.1038/nature10440.
[104] M. C. Motta et al., «Mammalian SIRT1 represses forkhead transcription factors», Cell, vol. 116, fasc. 4, pp. 551–563, feb. 2004, doi: 10.1016/s0092-8674(04)00126-6.
[105] M. Viswanathan, S. K. Kim, A. Berdichevsky, e L. Guarente, «A role for SIR-2.1 regulation of ER stress response genes in determining C. elegans life span», Dev Cell, vol. 9, fasc. 5, pp. 605–615, nov. 2005, doi: 10.1016/j.devcel.2005.09.017.

[106] G. Garroni et al., «Adipose-Derived Stem Cell Features and MCF-7», Cells, vol. 10, fasc. 7, Art. fasc. 7, lug. 2021, doi: 10.3390/cells10071754.
[107] A. Brunet et al., «Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase», Science, vol. 303, fasc. 5666, pp. 2011–2015, mar. 2004, doi: 10.1126/science.1094637.
[108] J. M. Denu, «Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases», Trends Biochem Sci, vol. 28, fasc. 1, pp. 41–48, gen. 2003, doi: 10.1016/s0968-0004(02)00005-1.
[109] H. Vaziri et al., «hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase», Cell, vol. 107, fasc. 2, pp. 149–159, ott. 2001, doi: 10.1016/s0092-8674(01)00527-x.
[110] J. Luo et al., «Negative control of p53 by Sir2alpha promotes cell survival under stress», Cell, vol. 107, fasc. 2, pp. 137–148, ott. 2001, doi: 10.1016/s0092-8674(01)00524-4.
[111] E.-J. Kim, J.-H. Kho, M.-R. Kang, e S.-J. Um, «Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity», Mol Cell, vol. 28, fasc. 2, pp. 277–290, ott. 2007, doi: 10.1016/j.molcel.2007.08.030.
[112] R. Bakthisaran, R. Tangirala, e C. M. Rao, «Small heat shock proteins: Role in cellular functions and pathology», Biochim Biophys Acta, vol. 1854, fasc. 4, pp. 291–319, apr. 2015, doi: 10.1016/j.bbapap.2014.12.019.
[113] B. Dukay, B. Csoboz, e M. E. Tóth, «Heat-Shock Proteins in Neuroinflammation», Front. Pharmacol., vol. 10, ago. 2019, doi: 10.3389/fphar.2019.00920.
[114] S. Lindquist e E. A. Craig, «THE HEAT-SHOCK PROTEINS», Annual Review of Genetics, vol. 22, fasc. Volume 22, 1988, pp. 631–677, dic. 1988, doi: 10.1146/annurev.ge.22.120188.003215.
[115] D. A. Parsell e S. Lindquist, «THE FUNCTION OF HEAT-SHOCK PROTEINS IN STRESS TOLERANCE: DEGRADATION AND REACTIVATION OF DAMAGED PROTEINS», Annual Review of Genetics, vol. 27, fasc. Volume 27, pp. 437–496, dic. 1993, doi: 10.1146/annurev.ge.27.120193.002253.
[116] C. Georgopoulos e W. J. Welch, «Role of the Major Heat Shock Proteins as Molecular Chaperones», Annual Review of Cell and Developmental Biology, vol. 9, fasc. Volume 9, 1993, pp. 601–634, nov. 1993, doi: 10.1146/annurev.cb.09.110193.003125.
[117] S. Cruciani et al., «Metformin and Vitamin D Modulate Inflammation and Autophagy during Adipose-Derived Stem Cell Differentiation», Int J Mol Sci, vol. 22, fasc. 13, p. 6686, giu. 2021, doi: 10.3390/ijms22136686.
[118] K. Nakamura, K. Rokutan, N. Marui, A. Aoike, e K. Kawai, «Induction of heat shock proteins and their implication in protection against ethanol-induced damage in cultured guinea pig gastric mucosal cells», Gastroenterology, vol. 101, fasc. 1, pp. 161–166, 1991, doi: 10.1016/0016-5085(91)90473-X.
[119] M. OTAKA, «Induction of a 60-kDa heat shock protein and prevention of caerulein-induced pancreatitis by water-immersion stress.», Gastroenterology, vol. 106, p. A312, 1994.
[120] Y.-L. P. Ow, D. R. Green, Z. Hao, e T. W. Mak, «Cytochrome c: functions beyond respiration», Nat Rev Mol Cell Biol, vol. 9, fasc. 7, pp. 532–542, lug. 2008, doi: 10.1038/nrm2434.
[121] X. Liu, C. N. Kim, J. Yang, R. Jemmerson, e X. Wang, «Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c», Cell, vol. 86, fasc. 1, pp. 147–157, lug. 1996, doi: 10.1016/s0092-8674(00)80085-9.
[122] R. J. Youle e A. Strasser, «The BCL-2 protein family: opposing activities that mediate cell death», Nat Rev Mol Cell Biol, vol. 9, fasc. 1, pp. 47–59, gen. 2008, doi: 10.1038/nrm2308.
[123] Y. Y. Tyurina et al., «Mechanisms of cardiolipin oxidation by cytochrome c: relevance to pro- and antiapoptotic functions of etoposide», Mol Pharmacol, vol. 70, fasc. 2, pp. 706–717, ago. 2006, doi: 10.1124/mol.106.022731.
[124] M. Ott, V. Gogvadze, S. Orrenius, e B. Zhivotovsky, «Mitochondria, oxidative stress and cell death», Apoptosis, vol. 12, fasc. 5, pp. 913–922, mag. 2007, doi: 10.1007/s10495-007-0756-2.
[125] C. Garrido, L. Galluzzi, M. Brunet, P. E. Puig, C. Didelot, e G. Kroemer, «Mechanisms of cytochrome c release from mitochondria», Cell Death Differ, vol. 13, fasc. 9, pp. 1423–1433, set. 2006, doi: 10.1038/sj.cdd.4401950.

[126] D. A. Fletcher e R. D. Mullins, «Cell mechanics and the cytoskeleton», Nature, vol. 463, fasc. 7280, pp. 485–492, gen. 2010, doi: 10.1038/nature08908.
[127] S. Etienne-Manneville, «Microtubules in cell migration», Annu Rev Cell Dev Biol, vol. 29, pp. 471–499, 2013, doi: 10.1146/annurev-cellbio-101011-155711.
[128] D. Vorselen, F. C. MacKintosh, W. H. Roos, e G. J. L. Wuite, «Competition between Bending and Internal Pressure Governs the Mechanics of Fluid Nanovesicles», ACS Nano, vol. 11, fasc. 3, pp. 2628–2636, mar. 2017, doi: 10.1021/acsnano.6b07302.
[129] J. Graf et al., «Omics Studies of Tumor Cells under Microgravity Conditions», Int J Mol Sci, vol. 25, fasc. 2, p. 926, gen. 2024, doi: 10.3390/ijms25020926.
[130] S. Cruciani et al., «Tuning Adipogenic Differentiation in ADSCs by Metformin and Vitamin D: Involvement of miRNAs», International Journal of Molecular Sciences, vol. 21, fasc. 17, Art. fasc. 17, gen. 2020, doi: 10.3390/ijms21176181.
[131] R. Kumar et al., «Identification of serum sirtuins as novel noninvasive protein markers for frailty», Aging Cell, vol. 13, fasc. 6, pp. 975–980, dic. 2014, doi: 10.1111/acel.12260.
[132] F. Balzano et al., «Behavioral Changes in Stem-Cell Potency by HepG2-Exhausted Medium», Cells, vol. 9, fasc. 8, Art. fasc. 8, ago. 2020, doi: 10.3390/cells9081890.
[133] G. Garroni et al., «Effects of the MCF-7 Exhausted Medium on hADSC Behaviour», Int J Mol Sci, vol. 25, fasc. 13, p. 7026, giu. 2024, doi: 10.3390/ijms25137026.
[134] S. Cruciani et al., «Modulation of adipose-derived stem cell behavior by prostate pathology-associated plasma: insights from in vitro exposure», Sci Rep, vol. 14, fasc. 1, p. 14765, giu. 2024, doi: 10.1038/s41598-024-64625-0.
[135] S. Cruciani et al., «Metformin and vitamin D modulate adipose-derived stem cell differentiation towards the beige phenotype», Adipocyte, vol. 11, fasc. 1, pp. 356–365, dic. 2022, doi: 10.1080/21623945.2022.2085417.
[136] V. Basoli et al., «Melatonin and Vitamin D Interfere with the Adipogenic Fate of Adipose-Derived Stem Cells», Int J Mol Sci, vol. 18, fasc. 5, p. 981, mag. 2017, doi: 10.3390/ijms18050981.
[137] M. Dulugiac, L. Moldovan, e O. Zarnescu, «Comparative studies of mesenchymal stem cells derived from different cord tissue compartments - The influence of cryopreservation and growth media», Placenta, vol. 36, fasc. 10, pp. 1192–1203, ott. 2015, doi: 10.1016/j.placenta.2015.08.011.
[138] J.-Y. Kim, J.-C. Kim, J.-Y. Lee, e M.-J. Park, «Oct4 suppresses IR-induced premature senescence in breast cancer cells through STAT3- and NF-κB-mediated IL-24 production», International Journal of Oncology, vol. 53, fasc. 1, pp. 47–58, lug. 2018, doi: 10.3892/ijo.2018.4391.
[139] K. Kesely et al., «Identification of tyrosine kinase inhibitors that halt Plasmodium falciparum parasitemia», PLOS ONE, vol. 15, fasc. 11, p. e0242372, nov. 2020, doi: 10.1371/journal.pone.0242372.
[140] D. J. Baker et al., «Opposing roles for p16Ink4a and p19Arf in senescence and ageing caused by BubR1 insufficiency», Nat Cell Biol, vol. 10, fasc. 7, pp. 825–836, lug. 2008, doi: 10.1038/ncb1744.
[141] T. Kamijo et al., «Tumor Suppression at the Mouse INK4a Locus Mediated by the Alternative Reading Frame Product p19 ARF», Cell, vol. 91, fasc. 5, pp. 649–659, nov. 1997, doi: 10.1016/S0092-8674(00)80452-3.
[142] I. M. Conboy e T. A. Rando, «The Regulation of Notch Signaling Controls Satellite Cell Activation and Cell Fate Determination in Postnatal Myogenesis», Developmental Cell, vol. 3, fasc. 3, pp. 397–409, set. 2002, doi: 10.1016/S1534-5807(02)00254-X.
[143] C. M. Beauséjour et al., «Reversal of human cellular senescence: roles of the p53 and p16 pathways», The EMBO Journal, vol. 22, fasc. 16, pp. 4212–4222, ago. 2003, doi: 10.1093/emboj/cdg417.
[144] M. Milanovic et al., «Senescence-associated reprogramming promotes cancer stemness», Nature, vol. 553, fasc. 7686, pp. 96–100, gen. 2018, doi: 10.1038/nature25167.
[145] T. H. Cheung et al., «Maintenance of muscle stem-cell quiescence by microRNA-489», Nature, vol. 482, fasc. 7386, pp. 524–528, feb. 2012, doi: 10.1038/nature10834.
[146] K. H. Vousden e D. P. Lane, «p53 in health and disease», Nat Rev Mol Cell Biol, vol. 8, fasc. 4, pp. 275–283, apr. 2007, doi: 10.1038/nrm2147.

[147] S. Santaniello et al., «Melatonin and Vitamin D Orchestrate Adipose Derived Stem Cell Fate by Modulating Epigenetic Regulatory Genes», International Journal of Medical Sciences, vol. 15, fasc. 14, pp. 1631–1639, ott. 2018, doi: 10.7150/ijms.27669.
[148] J. Nunnari e A. Suomalainen, «Mitochondria: in sickness and in health», Cell, vol. 148, fasc. 6, pp. 1145–1159, mar. 2012, doi: 10.1016/j.cell.2012.02.035.
[149] S. K. Calderwood, A. Murshid, e T. Prince, «The shock of aging: molecular chaperones and the heat shock response in longevity and aging--a mini-review», Gerontology, vol. 55, fasc. 5, pp. 550–558, 2009, doi: 10.1159/000225957.
[150] F. U. Hartl, «Molecular chaperones in cellular protein folding», Nature, vol. 381, fasc. 6583, pp. 571–580, giu. 1996, doi: 10.1038/381571a0.
[151] E. A. Craig, J. S. Weissman, e A. L. Horwich, «Heat shock proteins and molecular chaperones: Mediators of protein conformation and turnover in the cell», Cell, vol. 78, fasc. 3, pp. 365–372, ago. 1994, doi: 10.1016/0092-8674(94)90416-2.
[152] D. D. Mosser e L. H. Martin, «Induced thermotolerance to apoptosis in a human T lymphocyte cell line», J Cell Physiol, vol. 151, fasc. 3, pp. 561–570, giu. 1992, doi: 10.1002/jcp.1041510316.
[153] C. Mailhos, M. K. Howard, e D. S. Latchman, «Heat shock protects neuronal cells from programmed cell death by apoptosis», Neuroscience, vol. 55, fasc. 3, pp. 621–627, ago. 1993, doi: 10.1016/0306-4522(93)90428-l.
[154] M. Jäättelä, D. Wissing, P. A. Bauer, e G. C. Li, «Major heat shock protein hsp70 protects tumor cells from tumor necrosis factor cytotoxicity», EMBO J, vol. 11, fasc. 10, pp. 3507–3512, ott. 1992, doi: 10.1002/j.1460-2075.1992.tb05433.x.
[155] M. M. Simon et al., «Heat shock protein 70 overexpression affects the response to ultraviolet light in murine fibroblasts. Evidence for increased cell viability and suppression of cytokine release.», J Clin Invest, vol. 95, fasc. 3, pp. 926–933, mar. 1995, doi: 10.1172/JCI117800.
[156] A. Samali e T. G. Cotter, «Heat Shock Proteins Increase Resistance to Apoptosis», Experimental Cell Research, vol. 223, fasc. 1, pp. 163–170, feb. 1996, doi: 10.1006/excr.1996.0070.
[157] D. D. Mosser, A. W. Caron, L. Bourget, C. Denis-Larose, e B. Massie, «Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis», Mol Cell Biol, vol. 17, fasc. 9, pp. 5317–5327, set. 1997, doi: 10.1128/MCB.17.9.5317.
[158] J. H. Ahn, Y. G. Ko, W. Y. Park, Y. S. Kang, H. Y. Chung, e J. S. Seo, «Suppression of ceramide-mediated apoptosis by HSP70», Mol Cells, vol. 9, fasc. 2, pp. 200–206, apr. 1999.
[159] M. Jäättelä, D. Wissing, K. Kokholm, T. Kallunki, e M. Egeblad, «Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases», The EMBO Journal, vol. 17, fasc. 21, pp. 6124–6134, nov. 1998, doi: 10.1093/emboj/17.21.6124.
[160] R. Njemini, I. Bautmans, O. O. Onyema, K. Van Puyvelde, C. Demanet, e T. Mets, «Circulating Heat Shock Protein 70 in Health, Aging and Disease», BMC Immunology, vol. 12, fasc. 1, p. 24, mar. 2011, doi: 10.1186/1471-2172-12-24.
[161] «Tissue-specific regulation of cytochrome c by post-translational modifications: respiration, the mitochondrial membrane potential, ROS, and apoptosis», doi: 10.1096/fj.201801417R.
[162] N. A. Bishop e L. Guarente, «Genetic links between diet and lifespan: shared mechanisms from yeast to humans», Nat Rev Genet, vol. 8, fasc. 11, pp. 835–844, nov. 2007, doi: 10.1038/nrg2188.
[163] V. Calabrese, C. Cornelius, A. T. Dinkova-Kostova, E. J. Calabrese, e M. P. Mattson, «Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders», Antioxid Redox Signal, vol. 13, fasc. 11, pp. 1763–1811, dic. 2010, doi: 10.1089/ars.2009.3074.
[164] A. Satoh et al., «Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH», Cell Metab, vol. 18, fasc. 3, pp. 416–430, set. 2013, doi: 10.1016/j.cmet.2013.07.013.
[165] P. Yan et al., «LARP7 ameliorates cellular senescence and aging by allosterically enhancing SIRT1 deacetylase activity», Cell Rep, vol. 37, fasc. 8, p. 110038, nov. 2021, doi: 10.1016/j.celrep.2021.110038.

[166] A. S. Lundberg, W. C. Hahn, P. Gupta, e R. A. Weinberg, «Genes involved in senescence and immortalization», Current Opinion in Cell Biology, vol. 12, fasc. 6, pp. 705–709, dic. 2000, doi: 10.1016/S0955-0674(00)00155-1.
[167] C. E. Xirouchaki et al., «Skeletal muscle NOX4 is required for adaptive responses that prevent insulin resistance», Science Advances, vol. 7, fasc. 51, p. eabl4988, dic. 2021, doi: 10.1126/sciadv.abl4988.
[168] M. Z. Nassef et al., «Real Microgravity Influences the Cytoskeleton and Focal Adhesions in Human Breast Cancer Cells», International Journal of Molecular Sciences, vol. 20, fasc. 13, Art. fasc. 13, gen. 2019, doi: 10.3390/ijms20133156.