Mammalian Extracellular Matrix and Microgravity

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The extracellular matrix (ECM) is a constituent of all tissues, representing an integrating network that consists of specific and similar attributes. The ECM always includes collagen and noncollagen proteins, glycoproteins, and proteoglycans. The molecular composition of these structures demonstrates a huge diversity determined by the functional needs of a particular tissue. Meanwhile, it is the structural uniformity that ensures the operation of the ECM as a putative gravity detector. It is reasonable to assume that ECM components can act as an extracellular “mechanical sensor”.

mechanoreception gravireception microgravity simulation extracellular matrix

1. Introduction

To date, the extracellular matrix (ECM) as a complementary counterpart of gravireception has been studied to a lesser extent than the cellular one. At the same time, the data from space flights and ground-based simulations clearly demonstrate that the skeleton with a well-represented ECM is most sensitive to the lack of gravitational loading [1][2][3]. It is obvious that further progress in the elucidation of the mechanisms of physiological adaptation to microgravity requires the analysis of gravisensitive unit components with a special focus on the involvement of ECM structures.

2. Extracellular Matrix and Microgravity

Gravity is a universal mechanical stimulus that determines the mechanical homeostasis of tissues on the Earth. Accordingly, the ECM tensegrity stabilizing elements, i.e., tension and compression, will be affected by gravitational unloading at all structural levels of the body. From this point of view, the skeletal system is most sensitive due to a significant amount of ECM.

Normal bone tissue homeostasis is based on the dynamic balance between two closely related physiological processes: the formation and degradation of the bone matrix [4]. This ensures the functional adaptation of bone tissue depending on the requirements of the “external mechanical field”, including the levels of loading support, as has been shown in vivo [5][6][7].

With the beginning of human exploration of space, the issue of microgravity effects on the bone system has become particularly important. To date, it is known that bone mass may decrease due to the attenuation of mechanical impulses with the lack of mechanical loading (hypokinesia, hypodynamia, or immobilization) [8]. It was assumed to occur due to the dysregulation of concomitant adaptive bone tissue remodeling. Similar deformations were supposed to occur under microgravity [2][5][9]. The changes in mineral metabolism and human bone tissue state during space missions have fully justified these theoretical prerequisites. The data set of the detected physiological changes included the loss of total bone mass, demineralization of bones bearing an axial support loading on the Earth, a negative calcium balance, and increased bone resorption markers in blood and urine [2][9][10]. These data convincingly indicate that atrophic bone tissue rearrangements do occur under microgravity, with their severity largely depending on the bone location relative to the gravity vector [2][11][12][13]. Obviously, this fact can significantly limit the duration of a human stay in space due to the potential risk of osteoporosis and be followed by adverse consequences upon the return to the gravity of Earth.

During manned space flights, special attention is paid to countermeasures to reduce the adverse effects of microgravity, which can attenuate the severity of changes in the skeletal system. Therefore, the examination of the effects of real and simulated microgravity in experimental animals is of particular interest because of the absence of preventive measures.

The opportunities for aboard animal experiments are extremely limited. Only a few of such studies have obtained data on ECM and its components. In rats, after 18–22 days at the Bion-M1 Cosmos biosatellite program, a reduced mineralization of bone tissue, an inhibition of periosteal and endosteal remodeling in trabecular bones and the vertebrae, a decreased number of osteoblasts, and an unchanged number of osteoclasts were observed [14][15][16][17][18][19].

A reduced level of collagen type I and the appearance of collagen type III typical of embryonic tissues and early stages of inflammation and healing were found in the bone tissue [20]. In STS-1 and STS-2 experiments, after 9–14 days of zero gravity, mice had initial signs of tibial osteopenia and inhibition of bone growth in length [18]. Downregulation of genes encoding bone tissue proteins, osteonectin, osteocalcin, and collagen type I in femoral bone cells was observed [21]. The exposure of murine embryonic bone tissue culture at the Kosmos-2229 biosatellite was followed by the rate reduction in mineralization with a simultaneous increase in the tissue resorption rate [22].

After a 30-day flight at the Bion-M1 biosatellite, significant alterations were found in murine cartilage tissues: a decrease in articular cartilage proteoglycans associated with the downregulation of genes encoding mechano-responsive and structural cartilage matrix proteins, fibromodulin (FMOD), osteoglycin (OGN), clusterin (CLU), decorin (DCN), collagen X (COL10A1), trombospondin-4 (TSP4), and cartilage oligomeric protein (COMP). In contrast, no such disturbances were found in the sternal cartilage. The authors emphasized that it was the weight-bearing articular cartilage and not the minimally loaded sternal fibrocartilage that was adversely affected by microgravity [23].

Aboard the ISS, live imaging of medaka fish demonstrated an excessive DsRed-osteocalcin glycoprotein fluorescence compared to the ground control in pharyngeal bone osteoblasts at flight day 1. At flight days 5 and 8, the increased fluorescent signal persisted. Post-flight HiSeq global gene analysis found upregulation of genes encoding matrix proteins of osteoblasts: OCN (osteocalcin) and collagen type X (COL10A1). Based on the above, the authors concluded that the transcriptional response to microgravity developed very rapidly [24].

In 2021, Fu et al. published the results of a meta-analysis of available data on space flight bone tissue changes in rodents and primates. All experiments reported a decreased bone mass, more significant in trabecular bones compared to the cortical bones. The bones that were weight bearing under normal gravity were affected to a greater extent than the minimally loaded ones. Changes in the lower extremity bones were much more pronounced than that in the upper ones. The rate of trabecular bone mass loss was almost independent of the flight duration and was −1.7% a day. In comparison, the authors indicated that this parameter was much lower for astronauts (0.7–2.7% per month) due to the countermeasures undertaken. Osteoblast indices in the rodent trabecular bone were significantly lower than in cortical bones, while the numbers of osteoclasts showed no changes in rats and varied in mice. Based on the meta-analysis, the authors concluded that microgravity causes a bone tissue deficiency in rodents and primates, which may be associated with the decreased activity of osteogenic cells [25].

The hind limb suspension model is the most common one for ground-based microgravity simulation experiments. In specially equipped cages, rodents are placed in such a way that the hind limbs are deprived of support, while the animals can move freely on their front paws and have unlimited access to water and food [26]. Rodent experiments of various durations (up to 28 weeks) demonstrated a significant decrease in the bone mass in the trabecular parts of the femur and tibia, similar to the effects of inflight experiments (1.1–3.5% a day). In addition, similar to during the flights, the changes in the trabecular parts of bones were more significant than in the cortical ones.

Local bone mass losses under mechanical load deficiency or microgravity suggest that a mechanical signal (or its lack) can also be perceived at the cell level. The stromal lineage cells represented both by differentiated and progenitor elements can be involved in the process.

In vitro, experiments of various durations using osteogenic precursors have been performed in several space flights. Both at the unmanned Foton-10 satellite [27] and in the manned STS-54 [28] and STS-59 [29] missions, a downregulation of *OCN*-encoded glycoprotein osteocalcin was detected from flight day 5 to day 12. In addition, experiments at Foton-10 and STS-59 demonstrated a decreased transcription of *COL1A2* (collagen type I). With increased flight duration, after 17 days on board STS-80, the expression of a number of osteogenesis-related genes (*ALPL*, alkaline phosphatase; *ON*, osteonectin; and *OCN*, osteocalcin) in human fetal osteoblasts showed no differences from ground controls [30].

Therefore, inflight observations are the basis to believe that microgravity adversely affects the transcription of genes encoding ECM proteins during up to 14-day flights. The increased fluorescent signal of DsRed-osteocalcin described in medaka fish may indicate the post-translational effects of microgravity [24].

To study the effects of gravity deprivation on cells on the Earth, various simulating equipment has been developed. These devices include 2D and 3D clinostats, a random positioning machine (RPM), and rotary wall vessels (RWV) [31]. The common feature of all ground-based devices is the randomization of cell position relative to the gravity vector. Such equipment provides the opportunity to elucidate the mechanisms of the altered hypogravity impact on cells and to adapt methodological approaches prior to their use in space missions.

Gravity vector randomization studies using 2D/3D clinostats and RPMs detected multidirectional changes in the transcription of genes encoding structural proteins, as well as of ECM-associated and affiliated molecules. Buken et al. (2019), after a three-day fibroblast RPM exposure, described an increase in *COL4A5* and transcription/translation of the major ECM glycoprotein, fibronectin *FN*/FN. In MC3T3-E2 osteoblasts, the transcription of genes encoding enzymes that provide extracellular post-translational modification of collagen fibers, *PLOD1* and *PLOD2*, and the functional activity of these enzymes were increased during clinorotation [32]. After 7 days of 2D clinorotation, an increase in the expression of *COL1* and *COL3* was observed in MSCs [33]. On the other hand, there is evidence of a decrease in the expression of *COL1A* and *FBN1* (fibrillin) after 7 day's aclinorotation [33][34]. Regardless of the model and exposure time, a downregulation of *OMD* (osteomodulin) that regulates adhesion was shown in the preosteoblasts [35][36]. In addition to changes in structural proteins, a decreased transcription of genes encoding molecules associated with ECM metabolism, transcription factors, *cbfa1/RUNX2*, and growth factors *BMP3, FGF4, GDF2*, and *GDF3* was observed [35][37].

The available data suggest the existence of complex time-dependent changes in the transcriptional activity of matrix-associated genes. After 5 days at RPM, the transcription of The available data suggest the existence of complex time-dependent changes in the transcriptional activity of matrix-associated genes. After 5 days at RPM, the transcription of COL1A1, COL2A1, and COL9A1 was downregulated in bone marrow MSCs. After 10 days, no difference was found, and after 20 days, a significant upregulation compared to the static control was observed. At the same time, after 10 and 20 days of exposure, the expression of OMD was reduced, and the expression of OCN had no differences vs. static control [35]. According to Ratushnyy and Buravkova (2017), after a 96-h of adipose MSC exposure at RPM, upregulation of COL12A1, COL15A1, COL16A1, COL1A1, COL5A1, and COL8A1 and the glycoproteins THBS1, THBS2, THBS3, LAMA, SPARC, TNC, VCAN, and VTN was observed. With a longer exposure (10 days), the number of ECM genes with an altered transcription decreased. An increase was found only in *COL11A1*, LAMB3, and *TN* [38].

In addition to the stromal cell transcriptional activity, several studies (cited above) analyzed the ECM features per se. Zhivodernikov et al. (2020) described a decrease in collagenous and an increase in noncollagenous proteins in MSC ECM after a 10-day RPM exposure [39]. After 12 days of MSC clinorotation, a decreased mineralization of the ECM was detected [40]. A similar effect was found after a 20-day RPM exposure of osteo-induced MSCs [35]. Interestingly, a long-term (20 days) RPM microgravity simulation had different effects on the mineralization efficacy of the ECM secreted by stromal cells of different commitments: calcium deposition was attenuated in osteo-committed MSCs, and, on the contrary, it was increased in osteoblasts [35].

The data from several studies clearly demonstrated the significant alteration in the transcription levels of matrisome-related genes in stromal lineage cells. This list includes genes encoding core matrisome compression-resistant proteins such as collagens (COL), laminins (LAMA, LAMB), as well as glycoproteins: trombospondins (THBS), fibrillin (FBN), tenascin (TNC), vitronectin (VTN), etc. In addition, the genes of the ECM-remodeling protein encoding lysyl hydroxylases (PLOD1, PLOD2) also changed transcription. Above glycoproteins, so-called matricellular proteins are involved in the regulation of tissue development, function, and regeneration by controlling cellular response. These molecules act as a depot of growth factors and other bioactive mediators. The retention/release of these molecules governs cell adhesion, migration, proliferation, differentiation, etc. [41]. The complex changes in transcription/production of matrisome components under microgravity no doubts modifies not only the gravisensitivity of cells, but their functions as well. Under short-term real or simulated microgravity, multidirectional changes in the transcriptional activity of ECM-associated genes were found to provide the adaptation of stromal lineage cells with the above impact. In general, the direction of these changes is independent of the commitment level of stromal precursors. The few observations regarding ECM features make it possible to conclude that the ECM content and its mineralization decrease as a result of microgravity.

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