MicroRNAs (miRNAs), the Final Frontier: The Hidden Master Regulators Impacting Biological Response in All Organisms Due to Spaceflight

Charles Vanderburg[1], Afshin Beheshti[1,2] *

[1]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA

[2] KBRWyle Labs, NASA Ames Research Center, Moffett Field CA, 94035, USA

*Corresponding Author:
Afshin Beheshti, PhD
NASA Ames Research Center
Moffett Field, CA 94035
email: afshin.beheshti@nasa.gov

Abstract

In the past few decades biological research related to travelling in space has been rapidly growing. The majority of this research is for one purpose, to identify risks to human health that can be caused by the space environment and potential methods to mitigate these risks through development of novel countermeasures. This research will assure safer travel for astronauts involved in current missions on the International Space Station (ISS) and future long-term deep space missions to the moon and Mars. Although such biological research projects have revealed interesting findings that can potentially assist with these missions, the majority of space biology researchers have ignored a key biological factor, the microRNAs (miRNAs) that have emerged as important drivers of biological processes in human health and disease. MiRNAs are a major type of small non-coding RNA (approximately 22nt in length) that have been shown to be regulators of protein expression acting at every step from transcription to translation. One miRNA has the potential to target groups of hundreds of genes. In this comprehensive review, we will cover the history of miRNAs and the biological processes of miRNAs, our systems biology view of miRNAs, and finally the existing knowledge of miRNAs related to space biology. We will discuss the potential use of miRNAs as biological dosimeters for space radiation, the specific role of miRNAs with regard to radiation and microgravity, and the impact miRNAs have on health risks associated with spaceflight.

1. Introduction

Throughout the history of the biological sciences, features once considered small and insignificant are eventually identified as major, and sometimes found to be central mechanisms behind fundamental biological processes. This evolution of scientific understanding typically results in the explosion of a new frontier at which we re-evaluate what we previously thought was fully understood. Until 1993, the classical view of biology was that DNA is the building blocks of life that is copied or transcribed to messenger RNA (mRNA). Once mRNA is formed through this process, it is involved in translation which is the process of the mRNA directing protein synthesis with the assistance of transfer RNA (tRNA). (**Figure 1A**). This view was held constant during the studies of many pathological conditions. Unfortunately, this view left out what was previously

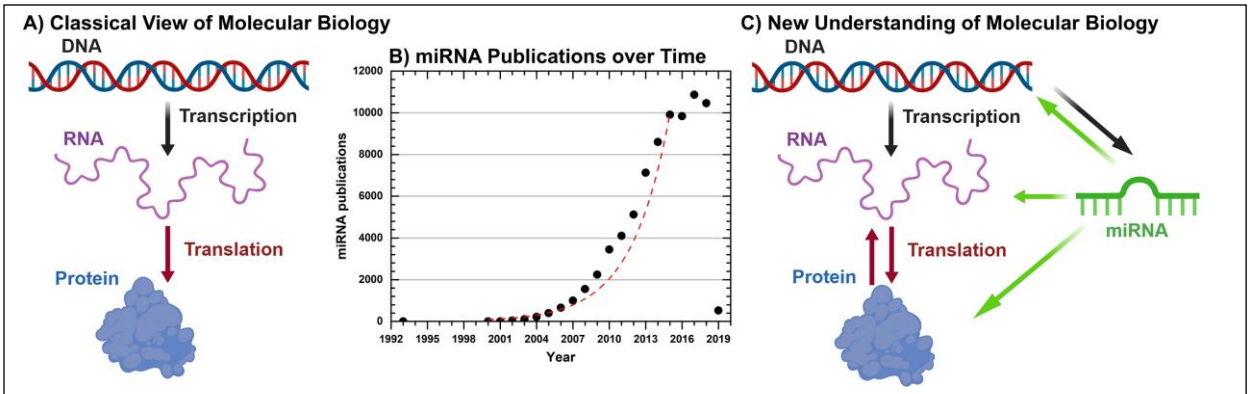

Figure 1. The new view for molecular biology related to miRNAs. A) Classical view of molecular biology. **B)** The miRNA related publications over time. Data was gathered from PubMed. The red dotted line shows an exponential fit to the data points. **C)** The new understanding of molecular biology based on miRNAs.

considered insignificant, namely small molecules of non-polyadenylated RNA, considered breakdown products, or transcripts of non-gene "junk" DNA.

In 1993, Victor Ambros was the first person to discover what was considered "junk" at that time to have some meaningful biological impact. Ambros et al. [1] discovered in *Caenorhabditis Elegans* (*C. Elegans*) that a 22-nt noncoding region identified as *lin-4* controlled the timing of larval development in *C. Elegans* by inhibiting the *LIN-14* gene. It was shown that the *lin-4* gene produced these 22-nt non-coding RNAs that had partial complementary sequences to the *lin-14* gene which caused the inhibition of the gene. At the same time a team led by Gary Ruvkun was able to establish additional information regarding *lin-4* by uncovering the molecular mechanism of how *lin-4* inhibits *LIN-14* [2].

Although these were very important discoveries for general biology it wasn't until 2000 when the next small RNA was characterized and skepticism from the community regarding its significance was put aside. Reinhart et al. [3] and Pasquinelli et al. [4] characterized and discovered that *let-7* directly inhibits *lin-41* in *C. elegans* to assist with the late stages of the development process. The importance of this is work is that it first implicated this class of small non-coding RNAs in a wide range of regulatory processes controlling gene expression in multiple species. It wasn't until a year later that Lagos-Quintana et al. dubbed these non-coding RNAs as microRNAs (miRNAs or miRs) and further demonstrated the highly conserved nature of the miRNAs between invertebrates and vertebrates [5]. Shortly thereafter, miRNAs gained attention in the scientific world as a newly identified class of regulatory RNAs conserved between different organisms that can target and regulate hundreds of genes [6-8]. Since then, there has been an exponential increase in reported miRNA-based biology related to many different types of diseases and organisms (**Figure 1B**) that has changed the classical view of biology (**Figure 1C**).

Delineating the production and regulation of miRNA isoforms is currently an evolving field, but some key steps have been identified and accepted regarding the process of miRNA synthesis. MiRNA sequences can reside in the intronic and exonic regions of both coding and non-coding genes [9, 10]. In addition, miRNAs can be single, bi-, or polycistronic and can be transcribed either dependent or independent of other genes [10-14]. Briefly, the biogenesis of miRNAs in eukaryotes is known to start with a nuclear phase which occurs via RNA polymerase II or III transcription of long primary RNAs (pri-RNAs) which are typically a few hundred nucleotides long [15, 16]. The pri-miRNAs are then cleaved to ~70 to 120-nucleotide hairpin shaped precursor

miRNAs (pre-miRNAs) by a multiprotein complex referred to as the Microprocessor primarily consisting of nuclear RNase III enzyme called Drosha and also DGCR8 [17, 18]. The pre-miRNAs are exported out of the nucleus by the protein exportin-5 [19, 20] and are then cleaved in the cytoplasm by an RNase III family member Dicer [21, 22] into ~22-29nt mature miRNA duplexes. The two strands are referred to either as the 5p (or 5′) or 3p (or 3′) strands. From the two strands that are formed one strand is called the guide strand and binds with catalytic Argonaute (AGO) proteins and creates a microribonuclear protein complex (miRNP) called RNA-induced silencing complex (RISC) [23-25]. The other strand is referred to as the passenger strand and is typically degraded [9, 24, 25], however, for some miRNAs the passenger strand can also bind with the RISC complex allowing both strands to be functional [26]. This miRNA-RISC complex is then guided to mRNA to regulate mRNAs. It was originally thought that miRNAs would only degrade or inhibit mRNA activity [14, 27], but it has been shown that miRNAs can also promote gene expression [10].

Due to this immense versatility of miRNA interaction, the classical view is thus revised to include the regulatory influence that miRNAs can have at each stage of gene expression (**Figure 1C**). A single miRNA may target 100s of genes and predictions have been made to estimate that up to 30% of all mammalian protein coding genes are regulated by miRNAs [28]. Thus dysregulation of the mRNAs provides reduced translation impacting the overall output of proteins, and it has been shown that such inhibition can cause up to 25% reduction of protein expression [14, 27]. In addition to the impact on protein expression and translation, miRNAs directly affect DNA. MiRNAs can hybridize with double-stranded DNA (dsDNA) to form triplexes leading to transcriptional gene silencing [14, 29]. It has also been reported that miRNAs can directly interact with single-stranded DNA (ssDNA) and directly impact the downstream transcription of target genes via chromatin modifications [14, 30]. It has even been shown recently that miRNAs are potentially the key drivers for DNA damage repair through miRNA-DNA hybrids [31]. Lastly, miRNAs can regulate DNA methylation and conversely DNA methylation can directly impact miRNA activity [32-36]. For example, through aberrant hypermethylation, miRNAs functioning as tumor suppressors are being down-regulated or silenced which increases malignancy and metastatic potential in breast cancer [32, 35]. Overall, the inclusion of miRNA biogenesis and function has revolutionized our view of molecular biology (**Figure 1C**). In this review we will tackle how miRNAs impact space biology and what we believe should be focus areas for future biological space research.

2. How miRNAs Relate to Space Biology

Astronauts are exposed to numerous health risks associated with long term space travel. It is essential to garner a comprehensive understanding of the mechanisms causing these health risks in order to develop strategies and countermeasures to ameliorate spaceflight associated disease. The environment that astronauts are exposed to in space consists of two major components that contribute to increased health risks: 1) space radiation which is comprised of galactic cosmic rays (GCR) that contain HZE ions (high (H) atomic number (Z) and energy (E) ions) and periodic solar particle events (SPEs) mainly composed of protons [37-42]; and 2) microgravity [43-46]. MiRNAs are increasingly becoming recognized as major systemic regulators of responses to stressors, including microgravity [46, 47], oxidative stress [48], radiation [49-52], and DNA damage [53, 54]. Some recent studies show the importance of miRNA signatures in response to radiation [49-52, 55-61], and studies involving low-LET radiation have demonstrated specific miRNA radiation-

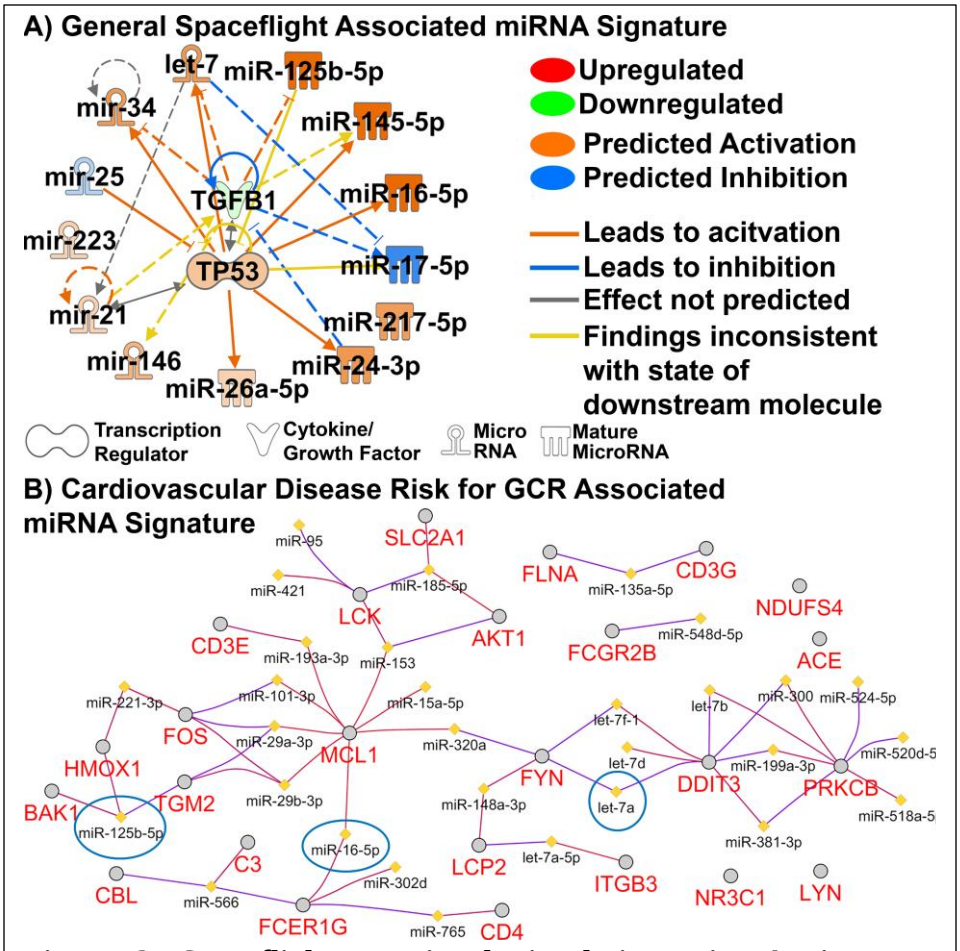

Figure 2. Spaceflight associated circulating miRNA signature predicted by Beheshti et al. [52, 63]. A) General spaceflight associated circulating miRNA signature. Figure adapted from Beheshti et al. [63]. **B)** Specific cardiovascular disease related miRNA signature. Blue circles represent miRNAs that overlap with **Figure 1A**. Figure adapted from Beheshti et al. [52].

dependent signatures. Yet little is known about the potential impact of miRNA and *space* radiation or microgravity since a direct assessment of miRNAs during human spaceflight remains to be accomplished. There has been a single study using a simulated microgravity model in humans [60] and none using spaceflight samples. Studying the direct influence of spaceflight on the miRNAs of astronauts will expand our general knowledge of human miRNA functions, allow us to evaluate miRNAs as potential minimally invasive biomarkers, and uncover specific miRNAs that could be genetically targeted for countermeasure development [62].

Using a systems biology approach, our previous work has shown that TGFβ signaling pathways are modulated by a spaceflight-associated miRNA signature, and hence impart critical immune response changes during spaceflight. Specific components of this miRNA signature are postulated to have direct impact on the cardiovascular system (**Figure 2**) [52, 63]. To determine and predict such miRNA signatures and their relationships, we utilized NASA's GeneLab platform (genelab.nasa.gov) [41, 64]. GeneLab is the first comprehensive platform to provide the public with omics data related to space biology. Although the system currently lacks miRNA-based omics data, the extensive GeneLab omics collection can still be used creatively to predict miRNA signatures via its suite of *in silico* algorithms and methodologies. We followed an approach utilizing transcriptomic GeneLab datasets of multiple tissues from mice flown in space to determine the first predicted miRNA signature associated with spaceflight (**Figure 2A**) [63]. Surprisingly, as described in Beheshti et al. [63], distinct miRNAs from the overall signature have been previously shown to be regulating specific functions in tissues due to spaceflight. For

example, miR-21 has been shown to be involved in T-cell activation during microgravity conditions [58]. Indeed, each miRNA in our overall predicted miRNA signature had previously been insinuated with some aspect of spaceflight response, but until the advent of our GeneLab analytics, the integrated picture has been elusive. In the next section we will further explore how a systems biology approach to studying miRNAs can provide an integral view of how miRNAs are truly involved in spaceflight. In addition to generalized spaceflight-associated miRNA profiling, we have predicted a miRNA signature specifically associated with cardiovascular disease risk due to space radiation (**Figure 2B**) [52]. In an independent analysis focused on datasets from GeneLab associated with cardiovascular disease, we were able to predict a second group of miRNAs being regulated in this system. From within this group we found three miRNAs that overlap with the initial spaceflight-associated miRNA signature, indicating that these three miRNAs may comprise the portion of the signature impacting cardiovascular disease [52]. In the following sections we will provide detailed information regarding how specific miRNAs will impact certain diseases and organisms associated with both microgravity and space radiation.

3. Systems Biology View of miRNAs

The majority of miRNA research tends to focus on just one or two miRNAs that are driving the system under study. Although focusing on a few factors does allow better understanding of how individual miRNAs impact the biology of an organism, the true association of how a biological system is impacted by all miRNAs is lacking. In this section we will highlight the importance of studying miRNA biology in groups rather as individuals – something that leads to better specific miRNA signature discovery in space biology.

We propose that utilizing a miRNA systems biology approach will provide a more accurate representation compared to studying miRNAs in isolation. We will use one of the first miRNAs discovered and one of the most studied miRNAs, let-7, as an example. When only considering let-7 as the miRNA of interest, a person can only observe that this miRNA contributes to decreased health risk (**Figure 3A**). Specifically, it has been observed that let-7 acts as a tumor suppressor when studied alone [65]. So, in that context a researcher would believe that an increase in let-7 expression decreases health risks. However, others have discovered that high-levels of let-7 lead to tumorigenesis by causing liver damage and degeneration [66]. This functional duality causes confusion – what is the biological relevance of this miRNA to disease in general?

To add another layer to the complexity of focusing on one miRNA at a time we consider situations where the same miRNA has been discovered to increase health risks for a certain disease while decreasing health risks for other diseases. Again, we turn to let-7 as example. In cancer, overexpression of let-7 has classically been thought to act as a tumor suppressor. But when broadening the search to include different diseases, one learns that overexpression of let-7 can impart increased risk of neurodegeneration through activation of Toll-like receptors [67]. And even in cancer, contradictory results have been published stating that let-7 can be a tumor suppressor or a promoter depending on the cancer type.

Since focusing on a single miRNA may provide misleading information on its overall impact on disease, how does studying two miRNAs at once change this view? For this example, we visit again let-7 and another well studied miRNA, miR-21. Similar to let-7, miR-21 has been shown to be involved in many different diseases, the overexpression of miR-21 showing both beneficial and negative health impacts [68]. The publication prevalence for each of these miRNAs alone would cause one to expect that there would also exist prevalent publications describing a relationship

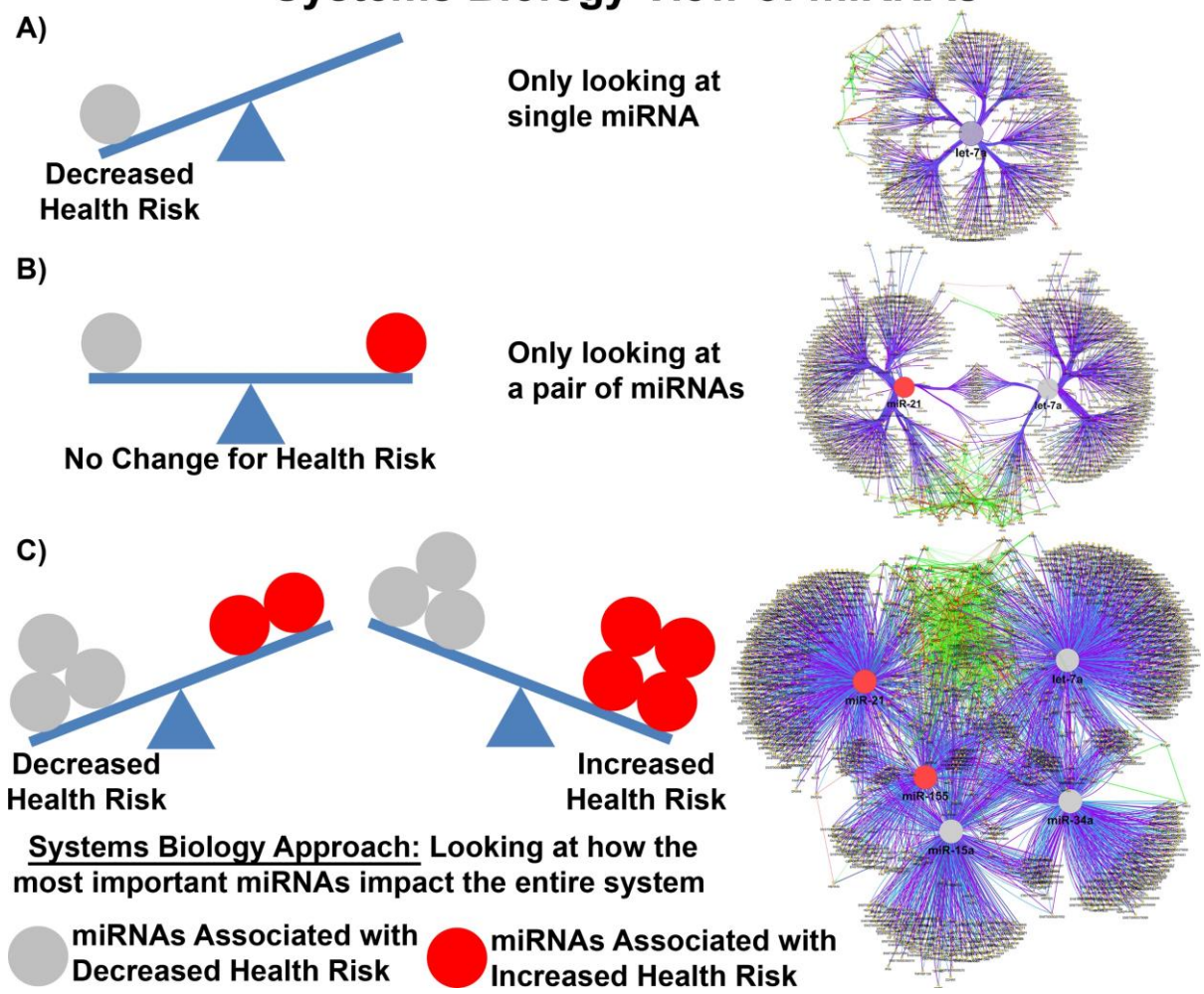

Figure 3. Systems biology view of miRNAs. Network figures generated with Cytoscape plugin ClueGo.

between these two miRNAs. But in fact, there are only a handful of publications that describe any direct impact of both miRNAs together [69-71]. We attribute this to the following, since the literature indicates that miR-21 and let-7 tend to have a reverse role in disease, these miRNAs studied together in isolation will most likely produce no change in health risks (**Figure 3B**). We hypothesize that any oppositely regulated miRNA set will basically negate any imbalance of the overall health risk based on the interactions with certain genes. This results when targets, genes, and/or molecular pathways are shared between two miRNAs, such as miR-21 and let-7 (**Figure 3B**). This again causes misleading results, especially in diseases where considering all factors both of these miRNAs share might be important, but with this view the importance will be missed.

This leads us to the systems biology approach which we consider the optimal way of viewing miRNAs related to diseases. (**Figure 3C**). We hypothesize that for any disease, stressor or environmental influence, there exists a miRNA signature consisting of multiple miRNAs that will function in unison to impact entire pathways and elicit systemic changes or disease progression. Using this ideology we have been able to determine a miRNA signature that is associated with lymphoma (specifically Diffuse Large B-Cell Lymphoma) [72], a general spaceflight associated

miRNA signature [63], and a predicted cardiovascular spaceflight associated miRNA signature [52]. The interactions between the group of miRNAs and the overall genes that will be affected, can be thought of as the key pathways for that certain disease or health risk. This view of how miRNAs function together will also explain why miRNAs in different contexts, such as different diseases, will behave differently. Since the same miRNA will interact with different groups of miRNAs for specific diseases, this will change the overall way the miRNA will behave when viewed in isolation in relation to that health risk. Once we consider how the rest of the miRNAs for that signature for the specific health risk interact with each other, then one can start to understand how these specific miRNAs will change. Utilizing this systems biology approach will allow us to fully understand how miRNA dysregulation impacts disease and has potential to clear up confusion on the complexity of individual miRNA changes.

4. MiRNAs Present in Many Organisms and the Association with Space Biology

The presence of miRNAs has been observed in all organisms. Within species, miRNAs are shown to be highly conserved functionally, but across kingdoms (i.e. miRNAs in plants and animals) evidence indicates that miRNAs are less conserved [73, 74]. Recent findings show that plant miRNAs enter mammalian systems through the bloodstream via the gastrointestinal tract to interact directly with the host's biology [75], an indication of conserved functionality of miRNAs within species and the possibly of differentially functional miRNAs impacting other organisms across species. Since ongoing space biology research is not only focused on human health (i.e. involving work done on vertebrates and invertebrates), but also includes studies of plants, microbes, and viruses, the miRNAs being transmitted within and between species can be important biology in a space environment. In this section we will briefly discuss the presence of miRNAs in different organisms and which organisms are poised for miRNA space biology research.

miRNAs Presence in Vertebrates for Space Biology Research

Although the first miRNA was discovered in *C. elegans* as described above, it was subsequently discovered that miRNAs play key roles in all vertebrates. MiRNAs exist in all mammals, birds, fish, reptiles, and amphibians, and the abundance of miRNAs in these different organisms exemplifies the important role it plays in vertebrates and their concomitant evolutionary biology. Vertebrate use in space biology research is primarily focused on humans and rodents [76] with occasional research that is also being done on fish (mainly zebrafish) [77-80]. Studies of vertebrate model systems miRNAs will be central to understanding molecular expression changes caused by the space environment since miRNAs can potentially be key drivers behind these changes. It has been shown that mature miRNAs are highly conserved in functions and sequences among vertebrates while the pri-miRNAs are not [81]. MiRNA space biology studies are primarily performed *in vivo* utilizing mice [55, 82], simulated microgravity experiments with humans referred to as bedrest studies [83, 84], or *in vitro* studies involving human cells [56-59, 85, 86]. Due to the conserved nature of miRNAs between mice and humans the majority of the rodent studies can be easily translated to human biological issues, but care should be taken in that mouse and human genes and disease are not always well correlated, especially in neural systems (see below).

miRNAs Presence in Invertebrates for Space Biology Research

In addition to vertebrate models, the primary organisms that scientists utilize for space biology related research are *Caenorhabditis elegans* (*C. elegans*) [49, 87-92] and *Drosophila melanogaster* (*D. melanogaster*) [93-97]. Both models are used to study factors related to human biology. For example, *Drosophila* is a model utilized to study immune related factors being regulated by the space environment [94, 95]. *C. elegans* is a decent model to provide a human analog for muscle related adaption to spaceflight, developmental biology in space, and space radiation [91]. Currently, miRNA related research done in invertebrates is primarily performed using *C. elegans* [49, 87, 90], these have focused on aspects of microgravity and space radiation causing apoptosis [49] and morphogenesis and development in the space environment [87]. Unfortunately, the miRNA research on invertebrates in space is rather limited, and besides these few *C. elegans* miRNA studies, there are no *Drosophila* related miRNA research studies reported. We will discuss below miRNAs involved with health risks and diseases and suggest possible future experiments that will be useful to conduct on invertebrates.

miRNAs Presence in Plants for Space Biology Research

Space biology research also includes studies of how various plants respond to the stress of spaceflight and of adapting plant growth to the space environment [98-104]. The majority of space biology plant research is performed on *Arabidopsis thaliana* [100-102, 104] which is a small flowering plant widely used as a model organism in terrestrial plant biology. The rapid life cycle, small genome, extensive genetic chromosomal maps, large seed productions, easy cultivations, and its ability to self-pollinate and cross-pollinate provides advantages that make *Arabidopsis thaliana* the optimal model organism to study plant biology in orbit [105, 106]. Interestingly, the first miRNAs associated with plants were discovered in *Arabidopsis* in 2002 by Llave et al. [107], Park et al. [108] and Reinhart et al. [109]. The differences between plant miRNAs and miRNAs of vertebrates and invertebrates is that plant miRNAs have a near-perfect complementarity to their mRNA targets [110]. This feature of plant miRNAs leads to increased target mRNA abundance, more efficient bonding with the target mRNAs to breakdown/silence the mRNAs, and increased confidence of miRNA target identification and validation [106, 109, 110]. In addition, similar to miRNAs in animals, plant miRNAs are highly conserved between different species of plants [110, 111].

Although miRNAs are heavily studied in terrestrial plant biology, in the field of space biology there is a distinct lack of research being done on the miRNAs of plants grown in the space environment. A current literature search reveals but one study by Xu et al. studying the impact of miRNAs for simulated microgravity experiments on *Solanum lycopersicum* (commonly known as a tomato) [112]. Their study revealed that long-term simulation of microgravity on *Solanum lycopersicum* resulted in response of miRNAs targeting genes involved in transcription regulation, signal transduction, and stress response. In addition, one of the key identified miRNAs, miR159e, demonstrated an accumulation of starch under microgravity conditions. Lastly, seven of miRNAs identified to response to stress under microgravity conditions were conserved in other plants. Xu et al.'s research provides a good example of the influence that miRNAs have on plants during life in the space environment, and the highly conserved nature of plant miRNAs lends itself to finding universal responses for miRNAs driving plant biology in space. Further research must be conducted to extend such findings.

miRNAs Presence in Microbes for Space Biology Research

How the space environment impacts microbes is also a burgeoning area of research for space biology. Ongoing studies examine microbes which are currently growing on the International Space Station (ISS) [113-116], how the microbial flora in humans change due microgravity and space radiation components [117-119], and potential ways microbes can be used as countermeasures to mitigate the space-related health challenges (i.e. probiotics) [120, 121]. One astronaut health concern is changes to the gut microbiome in response to long-duration spaceflight. It has been discovered that there is an increase in the relative abundance and complexity of the gut microbiome during spaceflight [122], which is hypothesized to be caused by the diet the astronauts consume. Conversely, it was also observed that there are decreases in beneficial microbes or commensals, such as *Lactobacillus* - this raises concerns about the overall health of astronauts on long-term space missions [123]. In addition, it has been reported that the space-modified gut microbiome may not recover within 60 days after return to earth [123, 124]. It is know that alterations of the healthy human terrestrial microbiome can lead to numerous health risks including but not limited to diabetes, anxiety, cancer, and infections [122, 125]. More research is required to fully understand whether spaceflight-associated gut microbiome changes indicate dysbiosis, or are tied to adverse health outcomes, but potential dysbiosis resulting from these shifts is currently of great concern.

It has been reported that miRNA's have direct impact on the gut microbiota by modulating the microbiota composition [126, 127]. Recent work has shown that miRNAs are able to enter gut bacteria and co-localize with the bacterial nucleic acid and that abundant amounts of miRNAs are present in fecal bacteria [126]. The influence of these exogenous miRNAs on gut bacteria results in direct regulation of gene expression and modulation of proliferation [126, 127]. The mechanism and process of these miRNA's entry into gut bacteria and the origin of the fecal miRNAs is not understood. The relationship between miRNAs and the gut microbiota becomes more complex with consideration of host diet. Changes in diet are known to impact the composition of the gut microbiota [128], which utilizes compounds that are unavailable to host metabolism and converts them into metabolites that may affect multiple factors of host physiology [129, 130], including miRNA expression. For instance, the short chain fatty acid butyrate, a product of fiber metabolism by species in the gut microbiota, was shown to inhibit the pathway for expression of an oncogenic miRNA in a colorectal cancer model [131]. A few recent studies have even shown an association between ingestion of specific probiotic bacterial strains with modified miRNA expression and improved gut microbiota composition in mouse models of colitis [132, 133].

With this complex interaction between miRNAs and microbes and the potential impact that spaceflight has on the overall microbial flora in humans, it is warranted to advance our understanding of the space environment's impact on miRNA infractions with microbes. Currently, there is a lack of direct research being performed in this arena. To demonstrate the potential influence of miRNAs on microbes we utilized our predictive tools and techniques [52, 63, 72] and predicted from our spaceflight associated miRNA signature (**Figure 2A**) the potential impact it might have on microbial activity and function. We are able to determine that the spaceflight associated miRNA signature including the key gene targets (TGβ1 and p53) [63], effect microbial related functions (**Figure 4**). The miRNA signature seems to be inhibiting bacteria death and invasion along with associated inflammatory and immune responses. In addition, we predict that enteritis should be strongly activated. Enteritis is a gastrointestinal disease that promotes

inflammation of the small intestine and is known to be caused by microbial changes in the gut [134-136]. It has also been shown that radiation treatment for diseases like cancer induces radiation enteritis by causing a selective imbalance of the gut microbiota due to differential radiation sensitivity [134]. Although miRNAs have not yet been investigated in association with enteritis resulting from radiation treatment, the evidence here suggests a link between them. Our predictive data provides a compelling association between miRNA profiles, gut microbiota and human health that could be important on earth as well as in spaceflight.

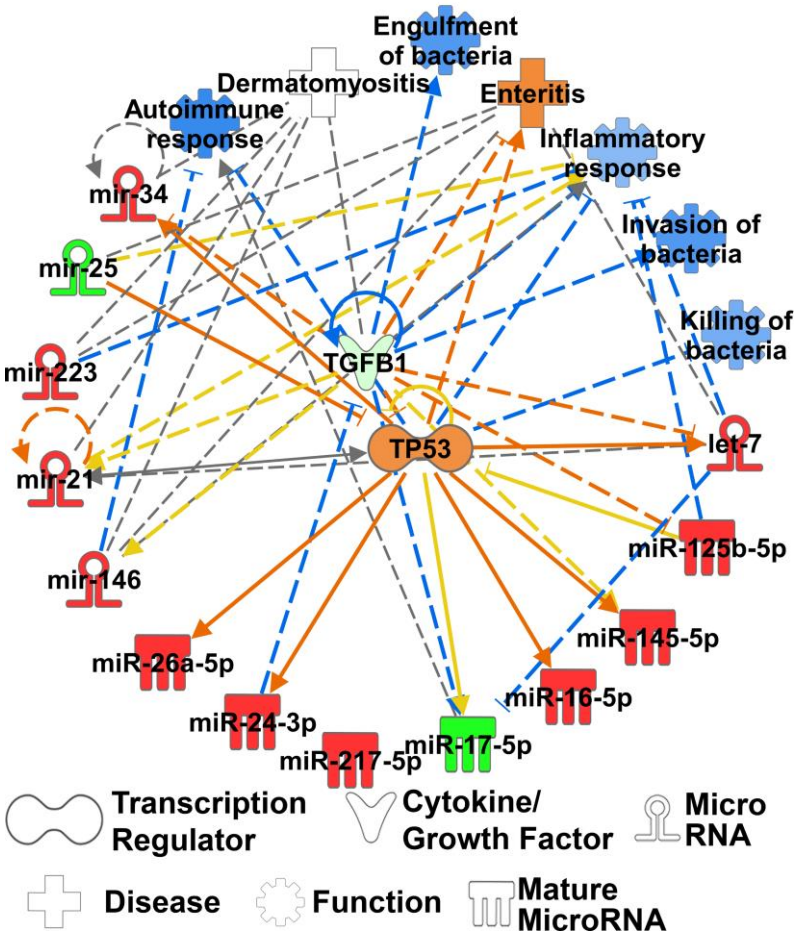

Figure 4. Circulating Spaceflight miRNA signature predicted impact on microbial related functions. Predictions were made using Ingenuity Pathway Analysis. Red = upregulated miRNAs, Green = downregulated miRNAs, Orange = activated pathways, Blue = inhibited pathways, and Yellow = prediction is contrary to what is known in the literature.

miRNAs and Viruses for Space Biology Research

Human space medicine research has revealed that significant changes occur in the immune system due to the space environment (specifically immune suppression) [46, 83, 94], Such changes are reported to be causative in the reactivation of dormant viruses such as Varicella Zoster Virus (also known as chickenpox) which is one of the eight known human herpes viruses [137]. These viruses typically remain inactive in immune cells throughout a person's lifetime. Rooney et al., has shown that due to the immune alterations occurring during spaceflight there is a high rate of reactivation of these herpes viruses. This is, of course, particularly concerning in astronauts who need to avoid passing any of these reactivated viruses to unaffected crewmates and/or the virally-associated problematic health issues during long-term space missions [137].

Due to the dearth of research on viruses and space biology, there currently is a lack of information concerning how miRNAs might be directly involved with this viral reactivation problem. However, the terrestrial research done on the interactions between miRNAs and viruses have revealed a complex interaction, and perhaps some hints. Specifically, viruses have been shown to avoid the immune response by making use of cellular miRNAs to finish their replication

cycles [138]. The following mechanisms have been shown to be central in the interaction of viruses and miRNAs: 1) miRNA pathways are blocked or inhibited by viruses interacting with key proteins [139], 2) viruses may employ specific target mRNAs to avoid or dysregulate cellular miRNAs [140, 141], 3) viruses can utilize miRNAs to redirect regulatory pathways to other miRNA targets to provide survival advantages [142], and viruses can encode miRNAs to produce viral miRNAs with well-defined functions to specifically target and regulate functions related to that virus [143, 144]. The reports of interplay between herpes viruses and miRNAs provide the best examples of how miRNAs are utilized by herpes virus to avoid the immune system and advance infections and disease [138, 140, 145]. Although, there isn't currently any research focused on miRNA-viral themes during spaceflight, the existing literature outside of space biology indicates that more attention should be given to this subject.

5. Circulating miRNAs and the Implications for a Minimally Invasive Biomarker for Diseases and Spaceflight Associated Health Risks

Due their small size and enhanced stability, miRNAs can circulate throughout the body and they have been found in the majority of the bodily fluids including blood, urine, saliva, and tears [146, 147]. These circulating miRNAs, also often referred to as extracellular or cell-free miRNAs, can be found either packaged in exosomes and other micro vesicles or due to their robust properties can also be freely floating in bodily fluids [148-151]. In the past decade, research has revealed that approximately 10% of miRNAs are sequestered in exosomes (or vesicles) and the other 90% are free floating in the fluid packaged with other proteins such as Ago2, high density lipoproteins, and other RNA-binding proteins [149-153]. It has been reported that unlike the highly unstable nature of mRNAs, miRNAs are amazingly stable in plasma, serum, and other bodily fluids with resistance to RNase activity, extreme pH, and multiple freeze-thaw cycles even after remaining 24 hours at room temperature in the specific fluid after extraction [148].

These properties of circulating miRNAs make them ideal candidates for a simple blood/bodily fluid-based biomarker to identify disease initiation and progression. Researchers are already demonstrating the powerful potential of utilizing circulating miRNAs to easily detect diseases and health status in cancers, cardiovascular disease, and muscle related issues [149-152, 154-156]. We have demonstrated the utility of cataloging circulating miRNAs in both cancer [72] and microgravity models [63]. Recently, through a systems biology approach, we were able to show that a distinct circulating miRNA signature in the blood significantly impacts disease development with and without microgravity [63, 72] and we have further data showing a component of this microgravity-induced circulating miRNA signature also impacts cardiovascular disease risk due to spaceflight [52]. Others have similarly reported distinct miRNA signatures in the serum that are associated with many different diseases and health risks. For example, it has been shown that specific miRNAs are found in the tears which are associated with eye disorders [157-159] and that hence specifically circulating miRNA profiles can be used as a biomarker for diabetic retinopathy [158]. Others have shown that circulating miRNA profiles in cardiovascular disease can be used as novel biomarkers indicative of disease state [150, 160-163] or as biomarkers of muscle degeneration [164-166] both with and without radiation damage. It has also been shown that distinct miRNA signatures for tumor burden arise in the serum and differ with and without radiation damage [160, 167-169]. Together, these findings support a hypothesis that there exists a distinct circulating miRNA signature that can affect the entire host from its organs to its tumors. Additional supporting research exists (mostly in solid tumors) for circulating miRNAs in cancer

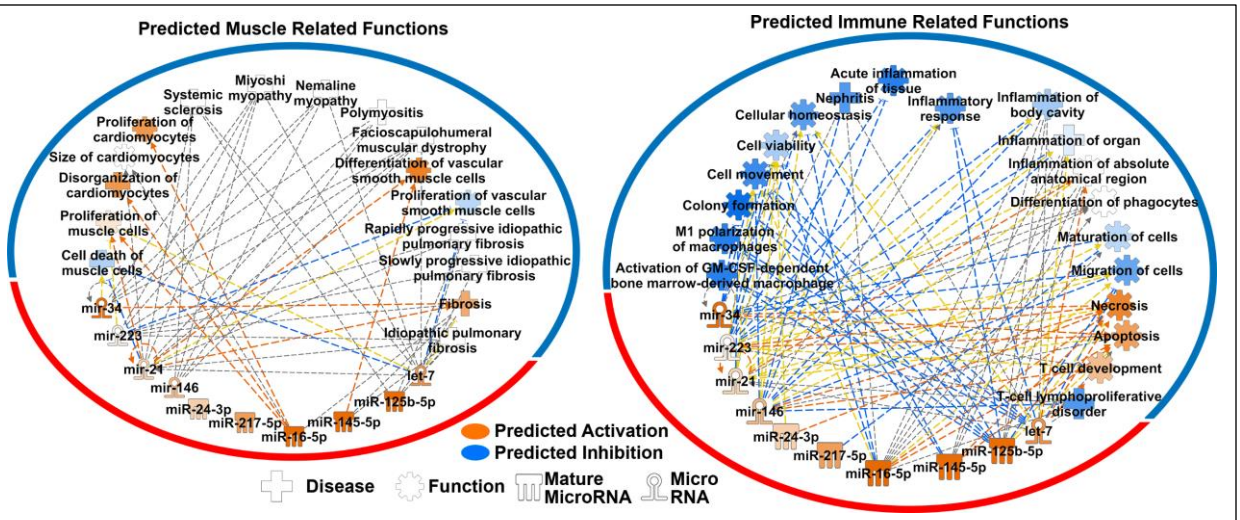

Figure 5. Circulating Spaceflight miRNA signature predicted impact on muscle and immune related functions. Predictions were made using Ingenuity Pathway Analysis. Red = upregulated miRNAs, Green = downregulated miRNAs, Orange = activated pathways, Blue = inhibited pathways, and Yellow = prediction is contrary to what is known in the literature.

risk for specific organs, but the where and how and which miRNAs affect non-disease related organs is not yet well understood. Substantial investment continues in the scientific and biotech communities to determine how such circulating miRNA profiles indicate or modulate health risk and disease progression.

Again, circulating miRNA-specific spaceflight-associated health risks are largely unexplored beyond our work to predict a circulating miRNA signature associated with spaceflight health risks (**Figure 2**) [52, 63]. As we discuss in Beheshti et al. 2018 [63], such spaceflight associated circulating miRNA signatures should be highly relevant to astronaut health, a concept that is borne out by the fact that the miRNAs in our discovered signature control specific biological functions and pathways that have regularly been reported as dysregulated during spaceflight (**Figure 5**). Overall, circulating miRNA profiles comprise excellent biomarkers for monitoring and assessing spaceflight associated health risks before, during, and after space missions.

6. MiRNAs Hidden Role with Radiation Damage and It's Role in Health Risk Assessment

MiRNA signatures are also associated with radiation responses [61]. A bystander organ-level response to low-LET radiation has shown a sex-specific deregulation of miRNAs in non-exposed spleens [170]. Studies involving low-LET radiation have demonstrated specific miRNA radiation-dependent signatures [61], but little is known concerning the potential influence of miRNAs, higher-level space irradiation, dose-rate effects or how the miRNAs function differentially in organs/systems or body-wide. Radiation effects have been reported in association with miRNA changes, and there are distinct tumor burden-associated miRNA signatures in the serum that arise differentially with and without radiation damage [160]. This indicates that there exists a distinct circulating miRNA signature that affects the entire host and that it originates from the organs most modulated by radiation exposure. Understanding how responses in the body change systemically as a function of space radiation and the potential use of the germane miRNAs as novel

biodosimeters are discussed in this section.

Sex Specific miRNA Response from Radiation Exposure

Before we describe in detail the specific impact miRNAs have during radiation response, we will briefly describe the sex differences that can arise with miRNAs during spaceflight and radiation exposure. It has been becoming more evident that biological sex differences are an important consideration when studying health risks associated with spaceflight [171-173]. Unfortunately, there currently is a lack of knowledge on specific sex-dependent miRNAs associated with exposure to the space environment. In addition, limited knowledge currently exists on sex-dependent miRNA response to general ionizing radiation. The current literature has indicated with low-LET radiation that there are specific miRNAs that are associated with by-stander effects in the spleen as mentioned above [170]. There has also been evidence that there are sex-specific miRNAs being regulated during radiation exposure to the brain. Specifically, the miRNA-29 family has been discovered to alter DNA methylation levels as a sex-specific epigenetic alteration in the hippocampus, cerebellum, and frontal cortex after low-LET radiation exposure [174]. Although there are some promising results demonstrating the miRNA sex-specific differences that occur with low-LET radiation exposure, at the time of this writing, no current literature exists on miRNA sex-specific differences that potentially can occur during space radiation exposure. It is our opinion that current research with miRNAs should be pursued to study sex differences that will occur, which will reveal miRNA-based sex-specific biomarkers and potential targets for countermeasures.

Chromosome Aberrations Resulting from Radiation Exposure and miRNAs

Radiation exposure results in organism-wide chromosomal aberrations caused by DNA damage and DNA double-strand breaks (DSBs) misrepair [175-178]. These aberrations can result in cell death, mutations, creation of micronuclei, immune related changes, and neoplasia [175, 178, 179]. It has been suggested that monitoring chromosome aberrations in the blood after radiation exposure can be a good tool for radiation biodosimetry [178]. The relationship between miRNAs and chromosomal abnormalities have been investigated in the context of cancer, since one of the main health risks due to chromosome aberrations is carcinogenesis. Through these investigations it has emerged that more than half of miRNAs are located in genomic regions where the chromosomal abnormalities occur, indicating that the location of the miRNAs are extremely important [180, 181]. In many cancers, miRNAs associated with oncogenic or tumor suppressor activity are in genomic regions approximating key cancer-related elements [182]. Regulatory elements of miRNAs that are around chromosome translocation sites elicit overexpression of oncogenes such as MYC [183]. It has also become clear that miRNA loci naturally insert into rearranged regions of chromosomes in early stages of carcinogenesis [180, 184], and this linkage of miRNAs to chromosomal aberrations has exploited to enable miRNA-targeted cancer therapies which prevent genomic aberrations from occurring [180, 181, 184].

Since radiation exposure causes high rates of chromosome aberrations similar to that seen in carcinogenesis [178], it is easy to hypothesize that radiation-specific miRNAs might play a similar role with radiation-induced genomic aberrations as they do with cancer. Indeed, a recent study has revealed that radiation-induced miRNA dependent changes do impact the number of chromosome aberrations that occur and thus result in genetic mutations [185]. Specifically, Liamina et al.

demonstrated that a radioresistant leukemia cell line compared to a radiosensitive leukemia cell line had miRNAs being more scattered throughout chromosome with more variance in expression values after irradiation [185]. There has been previous evidence (mainly involving cancer studies) showing that expression of miRNAs concentrated in specific regions of the chromosome can be a sign of more aberrations and/or translocations occurring [181, 186]. By considering this study in the context of previous knowledge concerning miRNAs, chromosomal aberrations, and cancer, we can postulate that radioresistant cells and tissues will have higher expression of miRNAs highly localized in chromosomal regions that are related to cell cycle, DNA repair, and cell death pathways. Work by Surova et al., confirms that higher expression of miRNAs *does* occur in radioresistant cell lines after ionizing radiation in a lung cancer cell line [187]. We can hypothesize that since functional miRNA groups circulate throughout the body providing a pattern of expression associated with a certain disease or environmental influence (as elucidated in the previous sections), then those circulating miRNA groups serves as good indicators of chromosomal aberrations that are occurring after irradiation, resulting in a good biodosimetry method to monitor chromosome aberrations. If this hypothesis is proven out, it leads to a quick and easy application for monitoring risk associated with space radiation.

MiRNAs as a Predictor of Radiation Induced Health Risk and Potential Utilization for a miRNA Based Biological Dosimeter

Evidence indicating that circulating miRNAs can be a good biomarker via radiation biodosimetry [188], focuses primarily on low-LET irradiation (i.e. gamma irradiation) and is starting to show promising results. Cui et al. performed a study observing miRNAs in the plasma after mice received total body irradiation of 0.5Gy, 2Gy, and 10Gy at both 6 and 24 hours post radiation [189]. They were able to delineate a circulating miRNA signature in the plasma associated with changes occurring as a function of increasing dose. They discovered that while at 0.5Gy no negative health risks could be predicted from the miRNA signatures, at 2Gy the miRNA signature predicted myelosuppressive and immunosuppressive pathway upregulation and at 10Gy the signature predicted poor survival based on cytokine target dysregulation. Similar results were obtained by Templin et al. when studying miRNA expression in blood from radiotherapy patients 4 hours after exposure to total body irradiation with 1.25Gy x-rays [190]. They discovered 45 miRNAs that could potentially be useful in biodosimetry.

A few excellent studies focus on miRNAs that were observed to be induced by radiation exposure and can be utilized to monitor health risks [191-193]. In these studies miRNA-30 was correlated to CD34 and immune response after radiation [192] and a dose and time dependent decrease in miRNA-150 in the serum was correlated to lymphocyte depletion and bone marrow damage [193]. There has even been a study by Ahmad et al., that identified high expression of miR-15b-5p in head and neck carcinoma patients after exposure to radiotherapy and demonstrated a significantly longer relapse-free survival when compared to patients with low amounts of miR-15b-5p expression [194]. Although these studies don't yet present a universal overlap or consensus miRNA profile associated with systemic radiation they do commonly show that circulating miRNAs in both the serum and plasma can be utilized as a sensitive biomarker for radiation biodosimetry [160, 189, 191, 193]. Additional studies need to be performed before a consensus miRNA signature emerges, but there is strong indication that miRNA-based biodosimetry may be possible.

MiRNAs Related to Galactic Cosmic Rays and Solar Particle Events

There are promising data being produced showing that miRNAs have significant regulatory influence upon biological functions as function of space radiation [46, 49, 55, 57-59, 63, 84, 85, 87, 90, 195-197] and potential to be used as a novel space biology dosimeters to quickly and efficiently measure health risks associated with high-LET radiation. Since our adoption of a systems biology approach to determine spaceflight associated miRNAs, components of this miRNA signature have been recapitulated in other research. For example, from simulated microgravity and radiation experiments done on the ground on human lymphocytes, Girardi et al., found both let-7 and miR-27a overlapped with our miRNA signature [57]. From experiments done with *C. elegans* flown to space for 16.5 days and receiving a total measured dose of 1.92mGy of space radiation, the human ortholog for the miRNAs being regulated by spaceflight was able to show that again the let-7 family, miR-92a, and miR-34 provided overlap with our miRNA spaceflight signature with impact on apoptotic pathways [87]. Zhang et al., was able to show in human fibroblast cells flown to space for 14 days that the space environment impacts miRNAs in the let-7 family for proliferative human fibroblasts while the non-proliferative conditions did not significantly change miRNA expression when compare ground controls [197]. Utilizing serum from a mouse model with whole body irradiation with both carbon ions and X-rays to simulate space radiation, Wei, et al. demonstrated that both let-7a and miR-200b were the two most significantly regulated miRNAs in the serum after irradiation [198]. From these studies there seems to be a story emerging with a possible miRNA signature that can be used to identify health risks associated with high-LET radiation.

MiRNAs Utilized for Space Radiation Dose Response at an Organ and Tissue Level

Our systems biology approach assumed that the miRNAs being regulated due to space radiation will have a systemic impact on the entire body [63] since these miRNAs are circulating throughout the body and thus easily accessing tissues, organs, and cells. Conversely, the miRNAs emanating from those tissues, organs and cells can also freely circulate, and may thus change with time the expression of the miRNAs observed in the blood. In certain disease models this bimodal behavior of circulating miRNAs has been observed - mRNAs or genes and the miRNAs regulating them may or may not be expressed similarly in both the blood and organs. For example in a drug-induced liver injury model, it has been shown that miR-122 and miR-192 become highly enriched in both the liver tissue and plasma following an overdose of acetaminophen which can be utilized to demonstrate drug-induced liver injury for mouse models [199]. This work also demonstrates that this miRNA pair has a dose and exposure-dependent change in the plasma that highly correlates to histopathology of liver degeneration and can be detected significantly earlier than current techniques for detecting liver damage. There has been research done showing how miRNAs are released into the circulation from the cardiac muscle during heart failure or myocardial infarctions and the specific circulating miRNAs associated with this disease can potentially be utilized to determine systemic response to cardiac injury and drug response [200, 201]. Due to such high correlations between circulating miRNAs and organ response, researchers have also investigated how miRNA expression can be a relevant biomarker for allograft injury and function [202]. It has been shown that circulating miRNAs provide a good assessment for organ quality [202], direct representation of ischemia-reperfusion injury [203], acute rejection [204, 205], chronic allograft dysfunction [206], and transplant tolerance for the organs [203].

MiRNAs have been shown to be good biomarkers for organ response and effectiveness of radiotherapy and chemotherapy, even showing specificity for tumor types [207]. Menon, et al. demonstrated that specific circulating miRNAs can be used as a multi-marker panel to estimate the toxicity in specific organs due to acute radiation sickness as a function of dose [208]. Circulating miRNAs have also been identified to be a good marker for hematopoietic injury induced by radiation as a function of dose, while conventional methods did not provide the sensitivity that the miRNAs exhibited [160]. A study on whole body exposure of low-LET radiation with multiple doses revealed that there are organ specific circulating miRNAs that can reflect the dose-dependence toxicity that occurs in different organs [189].

The promising results that we have put forth [63] coupled with such compelling evidence from others in the field warrants future investigations of miRNAs as biodosimeters, chemosensors or other biosensors for specific organ or tissue responses.

7. The Impact of miRNAs with Microgravity Response

To fully understand the cumulative effects of the space environment on human biology, researchers need to not only study space radiation, but also microgravity. Ultimately, the synergy of both components of spaceflight must be studied together to provide an accurate representation of the biological changes that will occur in the body in space. Many decades of literature are dedicated to the study of microgravity and biology including human physiological systems, and unfortunately a thorough review of that deep resource is beyond the scope of our intent here. However, a small portion of that work has been directed toward assessment of miRNAs in organisms under microgravity-induced stress.

Simulated microgravity experiments are most commonly performed terrestrially using either *in vivo* models typically utilizing a mouse hindlimb unloading model [209, 210] or *in vitro* models utilizing clinostats or rotary cell culture systems which simulates microgravity for cell culture models [211-213]. Using these models, investigators have discovered that miRNAs are indeed heavily involved in the biological pathways and mechanisms in response to microgravity. Several studies focused on microgravity's well documented causation of bone loss and osteoblast differentiation, have revealed that miRNAs play an important role in microgravity induced osteoporosis. One recent study investigated osteoclasts in cell culture under simulated microgravity conditions, showed a group of exosomal miRNAs are released from osteoclasts due to microgravity and target genes and pathways related to osteoporosis [214]. Mir-494 was shown in another study to inhibit osteogenesis and promote bone loss under microgravity conditions which directly targeted BMPR2 and RUNX2 genes that are heavily involved in osteoblast differentiation [215]. Mir-181c-5p was found to play an important role in microgravity induced inhibition of osteoblasts and increased bone loss by inhibiting factors related to cell cycle such as cyclin B1 [216] and Mir-132-3p has been implicated in bone remodeling under microgravity by inhibiting osteoblast differentiation through targeting of the Ep300 gene [217]. Microgravity is also known to induce cardiac and skeletal muscle atrophy and extensive studies have shown that certain miRNAs are heavily involved in targeting myotropic pathways and genes [218]. Several studies have also demonstrated that miRNAs regulate and directly target pathways in lymphocytes and lymphoblastoid cells under simulated microgravity conditions indicating miRNAs possible role with microgravity immune response [57, 59, 219]. Simulated microgravity experiments are also done in humans utilizing head-down tilt bed rest models and have revealed involvement of several circulating miRNAs involved with cardiac function and aerobic activity [220].

Specifically, Ade and Bemben [220] show for the first time that circulating miRNAs under simulated microgravity stress in humans contribute to upregulation of cardiovascular health related issues involving physiological and pathological cardiovascular remodeling. So, in general there is compelling evidence that miRNAs play an important role in the biological response elicited by microgravity. A full understanding of the systemic impact of miRNAs in spaceflight will require combining both microgravity and space radiation models, something that has yet to be accomplished experimentally.

8. MiRNAs Role in Increased Health Risks Associated with Spaceflight

In the previous sections we have cataloged the experimental evidence for miRNA-mediated effects associated with both microgravity stress and space radiation. In the sections that follow, we will cover in more detail the systemic and physiological changes occurring in astronauts during space flight, and establish hypothetical links between these and miRNA function.

Immune Changes that Occur in Spaceflight Regulated by miRNAs

There is mounting evidence of immunological dysregulation among the astronauts, such as the altered production of cytokines [221], enhanced sympathetic neuroimmune responses [222], compromised functions of monocytes [223], suppressed cytotoxicity of T-cells and Natural Killer (NK) cells [224], and reduced phagocytic capabilities of neutrophils [225]. Indeed, it has emerged that the alteration of glucocorticoid-mediated immune responses are well-correlated with spaceflight duration and that adaptive immunity shows high vulnerability to microgravity while some aspects of innate immunity are amplified [226]. Such immune function-related factors impact many organ systems. Abnormal production of immune cells in bone marrow disturbs bone homeostasis, osteoclast activities and bone resorption. Also, chronic inflammation negatively impacts modeling and remodeling of the musculoskeletal system. Related to neurological issues, as described by D´Mello and Swain, there are four main potential peripheral communication pathways to the brain that have been described in the setting of systemic inflammation: 1) the neural pathway, 2) signaling via cerebral endothelial cells, 3) signaling via the circumventricular organs, and 4) immune cell infiltration [227, 228]. These communications can be heavily disturbed in the event of spaceflight induced immune dysregulation. Furthermore, immunological dysregulations during spaceflight are likely compounded by significant alterations in skeletal physiology under microgravity conditions, as bone-forming osteoblasts play an increasingly appreciated role in support of lymphocyte development and function [229-231]. It has been shown in mice and aging men that bone loss is associated with reduced lymphocyte counts [229, 230, 232] and bone mass decreases dramatically during spaceflight [233, 234]. Disrupted immune health has long been recognized as an important factor in the pathogenesis and pathophysiology of cardiomyopathy. Higher abundance of circulating cytokines holds causal relationship with depressed myocardial contractility and high risk of heart failure. An elevated load of such cytokines, trigger the production of nitric oxide (NO), which prompt a rapid recruitment of pro-inflammatory cytokines and thereby initiates a vicious feedback loop. This loop eventually implicates inotropism and heightens myocardial damage [235, 236].

Evidence suggesting polymorphic characteristics of miRNA-mRNA interactions [237] endorses miRNAs as distinct posttranslational modifiers. The miRNAs can influence a wide range of immune functions via suppressing the stability of targeted mRNA and causing immune

dysregulation and inflammation [238-241]. Specifically related to spaceflight, Hughes-Fulford et al. have shown that miR-21 was significantly expressed in human leukocytes that were flown to the ISS and were directly targeting and regulating T-cell activation [58], and as highlighted in the previous section there are additional studies that demonstrate miRNAs are responsible for immune dysfunction through pathways involved with lymphocytes [57, 59, 219]. Chakraborty, et. al., demonstrated that when human endothelial cells were exposed to lipopolysaccharides on the ISS, miR-200 and miR-146b played an important role with oxidative stress and immunity in space [242]. With our spaceflight-associated miRNA signature we have made *in silico* predictions predicting immunosuppression (**Figure 5**) which agrees with the current literature. Further space-based research will delineate a specific miRNA signature that drives immune dysregulation during spaceflight.

Spaceflight Induced Cancer Risk and miRNAs

Cancer risk due in space is mainly driven radiation from Galactic Cosmic Rays and Solar Particle Events (which were previously described above). The majority of the studies on whether space radiation will induce carcinogenesis and progression of cancer are ground based simulated space radiation studies utilizing the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL) [243-245]. Utilizing their high energy beams, research has been done with single heavy ion radiation and recently with mixed heavy ion beams that closely match the composition of ions that an astronaut is exposed to in space [245]. Over the past decade, investigators have been able to identify several types of cancer that are considered to be high risk due to space radiation. These include breast cancer, lung cancer, leukemia, stomach, and ovarian with the risk of lung cancer considered to be the largest [246-252].

MiRNAs have been shown to mediate both immunity and carcinogenesis [54] and are increasingly used as biomarkers for cancer [72]. In the past decade many researchers have explored the potential of utilizing circulating miRNAs as a tool to assess cancer risk and progression monitoring during and after therapy [253]. Girardi et al. studied how miRNAs in human peripheral blood lymphocytes under microgravity conditions upregulated miRNAs involved with direct regulation of pathways that can potentially induce cancer risk [219]. A study done by Wang et al. to investigate the ideal mouse model to utilize for determining spontaneous lung carcinogenesis due to space radiation, revealed circulating miR-21 to be a key player [254]. Another study on human lymphoblastoid cells under simulated microgravity conditions revealed seven differentially regulated miRNAs that are highly associated with many different cancers and potentially can be used as a biomarker of microgravity induced cancer risk [59]. MiR-22 was shown to be significantly involved in colorectal cancer risk under microgravity stress, directly dysregulating pathways related to cell cycle and apoptosis [255]. Utilizing a systems biology approach, we identified a miRNA signature that is associated with patients with Diffuse Large B Cell Lymphoma (DLBCL) [72]. This, coupled with evidence from clinical data and emerging evidence from space biology is starting to reveal the important role that miRNAs have with cancer and the space environment.

Spaceflight Induced Cardiovascular Risk and miRNAs

Cardiovascular disease is a prominent concern in astronauts under spaceflight conditions [256-258]. Although experiments using low-LET (i.e. gamma or x-ray) experiments have been

conducted to study radiation damage to the microvasculature, charged particle and microgravity experiments are limited, and the implications of these risks need to be studied further.

Recently, distinct miRNA signatures in the serum have been identified in cardiovascular disease [150, 160-163] with and without radiation damage. Fuentes et al., studied the impact of simulated microgravity on neonatal and adult cardiovascular progenitors and discovered a group of miRNAs that had an age-dependent impact on cardiovascular disease [259]. Camberos et al., discovered in adult cardiac progenitor cells experiencing simulated microgravity conditions and also cells flown to the ISS, had upregulation of the Hippo pathway with YAP1 being a key driver in effecting repair in cells [260]. In their study they showed that miR-302a is directly responsible for inducing YAP1 expression and may be a useful countermeasure for protecting cardiac progenitor cells against spaceflight effects. Further work by others have shown other miRNAs including miR-16, let-7 family, miR-99a, and miR-100, targeting YAP1 and the HIPPO pathway on cardiac progenitor cells flown to the ISS which will impact cardiac development [85]. Ade and Bemben studied the impact of circulating miRNAs related to cardiovascular disease utilizing samples from head-down tilt bed rest subjects and discovered key circulating miRNAs that were associated with cardiovascular disease were upregulated under the simulated microgravity conditions (miRNAs included: miR-29a, miR-1, miR-30c, miR-126, miR-133a, miR-378a, miR-30b, miR-155, and miR-18a) [220]. We have delineated a group of miRNAs that are associated with cardiovascular disease risk in the space environment [52] and predict this signature will induce functional cardiomyocyte behavior that leads to disease. Overlap exists between our signature the Fuentes miRNA group, suggesting some possible miRNAs candidates for future cardiovascular risk studies.

Spaceflight Induced Muscular Degeneration and Bone Loss Risk and miRNAs

Skeletal muscle atrophy and bone loss is highly affected during spaceflight and is one of the major concerns for health risk for astronauts [261-264]. In addition, muscle and bone degeneration have been highly correlated and the interaction between these two tissues can be of major concern in microgravity [265, 266]. In the clinic, miRNAs have been shown to be intimately involved in bone related diseases, such as osteoporosis and malignant bone tumors [267-269] and miRNAs are known to regulate bone metabolism, homeostasis and remodeling via genesis and cellular differentiation of osteoblasts and osteoclasts, and skeletal development [268]. Circulating miRNAs have been identified that serve as ideal biomarkers for the osteoporosis [269] and these identified miRNAs can easily be implicated in bone loss due to spaceflight since the clinical diagnosis of osteoporosis is highly correlated [270]. miRNAs have also been highly correlated with muscle degeneration, atrophy, and wasting [271-273] and circulating miRNAs can be utilized as excellent biomarkers for muscle-related diseases and novel therapeutics [273]. There are proven relationships between circulating miRNAs which directly interact with bone disease and muscle wasting simultaneously [274, 275] as in the case of miR-34a which originates from the skeletal muscles as a function of age, increases in the circulation and directly induces senescence in primary bone marrow cells to contribute to increased risk of bone disease [275].

Currently, there are more miRNA related spaceflight reports regarding muscular health than there are for bone disease. For both muscle and bone related terrestrial experiments simulating microgravity conditions, two models are used: 1) in rodents the hindlimb unloading (or hindlimb suspension (HLS)) model [209, 210] and 2) for human ground based analogs, bed rest studies where an individual is in bed for weeks to months under a −6° head-down tilt bed rest [60, 276-

278]. Using HLS, Hu et al., show that miR-132-3p dysregulates osteoblast differentiation in rats inducing bone loss [217]. Similar studies identify six miRNAs that control osteoblast differentiation and proliferation [279], including Mir-139-3p which also induced osteoblast differentiation and apoptosis in murine HLS models with progression of osteoporosis [280]. Mir-208a-3p was another candidate for bone loss in another hindlimb unloading experiment which revealed that this miRNA inhibited osteoblast differentiation. Inhibition of miR-208a-3p thus upregulated osteoblast activity and mitigated the hindlimb unloading conditions [281]. From human bed rest studies, it was discovered that a panel of 10 circulating miRNAs were related to bone loss after 45 days of bed rest with miR-1234 demonstrating the major clinical relevance [60].

Experiments on muscle related miRNAs in spaceflight utilize the same experimental setups and often present results closely related to those for bone loss. MiRNA data exists from research done with rodents that have been flown to the ISS, something which does not currently exist for bone related research. Several reviews discussing skeletal muscle miRNAs identified a group of miRNAs associated with microgravity induced atrophy, inhibition of muscle growth, and muscle wasting [273, 282] and specifically, miR-206 is shown to be decreased in spaceflight samples [55]. A summary of hindlimb suspension experiments shows observed decreases in several other miRNAs (miR-107, -208b, -221, -499 and -23b) that result in atrophy, and several human bed rest studies show similar miRNA involvement via biopsy or circulating miRNA profiling [83, 283-285]. One of these studies demonstrated that the bed rest subject's atrophy is due to miRNAs influencing the immune system, specifically, miR-1-3p, miR-95-5p, let-7a-5p and miR-125b-5p have inflammatory roles, thus dysregulating skeletal muscle proliferation and differentiation as a downstream sequela [83]. Interestingly, two of these, let-7a-5p and miR-125-5p are part of our predicted circulating miRNA spaceflight signature (**Figure 2**) [63]. In another bed rest study it was discovered that miR-206, miR23a, and members of the let-7 family were driving skeletal muscle responses to inactivity [285] similar to the expected response human skeletal unloading in microgravity.

Spaceflight Induced Central Nervous System (CNS) Risk and miRNAs

In the past two decades, spaceflight has been shown to have significant impact on the central nervous system (CNS). This includes effects on normal cognitive abilities, behavioral changes, motor activity reduction, and an increase in CNS disease with Alzheimer's like impacts on short-term memory, dementia, and potential premature aging [286-288]. CNS related health risks in space have been attributed to both microgravity and space radiation. Microgravity has been shown to induce changes in cortical structure and function influencing motor functions and timing in astronauts [289]. Radiation certainly affects astronauts' cognitive abilities and causes behavioral issues, in addition to increasing risk of Alzheimer's disease [286, 288]. Anatomic and behavioral studies in mice have shown that the CNS has a unique susceptibility to space radiation exposure, with a degeneration of delicate neuronal structures that could lead to performance decrements and/or long-term sequelae [290, 291]. Clinical data from cranial radiotherapy from brain cancer patients showed that CNS radiation exposure can cause progressive and debilitating effects on cognition, including learning, memory, processing speed, attention, cognitive flexibility, executive function and behavioral disorders as increased anxiety, mood changes and depression [290]. Other clinical studies have also linked these effects to persistent neuroinflammation [290]. Although clinical data provided important insights for the CNS response to radiation, the complex space

environment with the presence of microgravity confounds the known clinical radiation effects [290].

Clinical data suggests that miRNAs play an important role in CNS related diseases with the potential of utilizing miRNAs as biomarkers and therapy [292-296]. It has been shown that miRNAs can drive neuroinflammation which can affect various neurological diseases such as Alzheimer's disease, multiple sclerosis, and ischemic stroke [297]. Research has also shown many miRNAs to be heavily involved in development and maintenance of normal neuronal function.[292]. This has led researchers to hypothesize that key miRNAs might be driving neurodevelopmental disorders, neuropsychiatric disorders, and neurodegenerative disorders. For example, miR-16 induces a depression-like phenotype in rats [298]. Mir-34a, miR-125b, and miR-146a (among other miRNAs) have been implicated in Alzheimer's disease [299-301]. These miRNAs have also been shown to be circulating in the blood and cerebrospinal fluid and researchers have started to realize the potential of these miRNAs as minimally invasive diagnostic biomarkers for various neurological diseases [294, 302, 303].

With regard to actual spaceflight model neural system research, not much yet exists. Khan et al., performed a whole body proton irradiation on mice simulating SPE irradiation and potentially identified eight miRNAs (miR-409-5p, miR-205, miR-100, miR-501-3p, miR-99b, miR-674, and miR-412-5p) being dysregulated in the brain of these mice compared to the controls [195]. Mir-21 was also identified in another study to be induced in the brains of mice exposed to whole body irradiation of ^{56}Fe ions and was found to target the EGFR pathway [304]. Neural miRNA profiles of microgravity models are non-existent, and as such, we currently have to rely on CNS related miRNA research to provide insight about CNS related health risks that might occur in space. As one example, 4 of the neurological disease related miRNAs mentioned above (miR-16, miR-34a, miR-125b, and miR-146a) have been shown to be dysregulated in our spaceflight associated circulating miRNA signature (**Figure 2**) [63].

Spaceflight Induced Spaceflight Associated Neuro-ocular Syndrome (SANS) and miRNAs

Spaceflight associated neuro-ocular syndrome (SANS) [305] has recently gained the attention of NASA as a potential health risk that can affect performance of astronauts in long-term space travel. It has been reported that astronauts on the ISS and also on space shuttle present with the following post-mission conditions associated with SANS: optic disc edema, globe flattening, choroidal folds, nerve fiber layer infarcts, thickening with the nerve fiber layer, and decreased vision [305-307]. The impact of SANS during missions is critical - decreased near visual acuity and on-orbit decreased distant visual acuity [305-307]. After return to Earth, the majority of astronauts gradually return back to their original vision with a few having minor residual effects [306, 307], although long lasting effects have yet to be correlated with exposure time and age. For a long-term space mission (i.e. trip to Mars), SANS may be prohibitive.

To date there is no clear understanding of what is causing SANS and how to remedy this health risk. It has been thought that SANS might be related to hypertension or microgravity affecting the cerebrospinal fluid in the orbital subarachnoid space due to elevated cerebrospinal fluid sheath pressures [305, 306]. Current knowledge is very limited on what the actual mechanisms causing SANS is and how what possible countermeasures can be used against it.

Research on miRNAs in ocular diseases such as glaucoma [157], diabetic retinopathy [158], cataracts, macular degeneration, and many other eye-related disorders [159] has been performed. Specific miRNAs are found in the tears which are associated with such eye disorders [157-159]

and a known circulating miRNA profile can be used as a biomarker for diabetic retinopathy [158]. We would, of course, hypothesize that there will be a correlation between miRNAs regulating SANS and a circulating profile or biomarker or therapeutic, and suggest that further research be done in this arena as well.

9. Conclusion

We have provided a comprehensive review of current space biology research focused on miRNAs. We have addressed the impact that miRNAs have in general on health risks associated with the space environment and explored their potential roles as biomarkers or therapeutics.

We briefly discussed the role of miRNAs in organisms other than mammals, but primarily focused on miRNAs associated with human health since the astronaut will be the ultimate model system for long term space travel. We hope we have convinced the reader of the importance of studying miRNAs in space biology research, adding that such endeavors may also enhance our understanding of terrestrial biomedicine.

As is apparent from this review, there are still many gaps to be filled, but the existing evidence from terrestrial disease-based miRNA research can be extrapolated to a vision of space-based correlates for multiple physiological systems. One major gap regarding miRNA research related to the space environment, is research involving miRNA response related to doses that will be received during a long-term mission to Mars [308]. The current literature referenced for this review related to miRNAs and space radiation, currently are either done at low Earth orbit on the ISS (i.e. with low space radiation dose exposures) or with single particle exposure acutely delivered and/or utilizing higher doses that an astronaut will experience on a mission to Mars. So, the current challenge with existing miRNA research in space biology is to extrapolate the result to accurate doses that an astronaut will be exposed to during long-term space missions. The new mixed ion fields now being offered at the NASA Space Radiation Laboratory (NSRL) [244] to provide more accurate simulation of GCR radiation for long-term space missions are a step in this direction. We recommend that future studies utilizing NSRL should incorporate miRNAs to fully characterize miRNA relevance to long-term missions in space.

We hypothesize that miRNAs have great potential to be used as a minimally invasive biomarkers for monitoring astronaut health during spaceflight. We also believe that in-depth future studies of miRNAs associated with spaceflight will unlock novel and cutting-edge countermeasures to mitigate the spaceflight effects on humans.

10. Acknowledgments

Research funding was provided by the GeneLab Project at NASA Ames Research Center, through NASA's Space Biology Program in the Division of Space Life and Physical Sciences Research and Applications (SLPSRA). Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government

11. Authors Contributions

C.V. provided extensive edits to the manuscript and exchanged ideas. A.B. wrote the manuscript and generated the figures.

12.References

1. Lee, R.C., R.L. Feinbaum, and V. Ambros, *The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14.* Cell, 1993. **75**(5): p. 843-54.
2. Wightman, B., I. Ha, and G. Ruvkun, *Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans.* Cell, 1993. **75**(5): p. 855-62.
3. Reinhart, B.J., et al., *The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans.* Nature, 2000. **403**(6772): p. 901-6.
4. Pasquinelli, A.E., et al., *Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA.* Nature, 2000. **408**(6808): p. 86-9.
5. Lagos-Quintana, M., et al., *Identification of novel genes coding for small expressed RNAs.* Science, 2001. **294**(5543): p. 853-8.
6. Ambros, V., *microRNAs: tiny regulators with great potential.* Cell, 2001. **107**(7): p. 823-6.
7. Moss, E.G., *MicroRNAs: hidden in the genome.* Curr Biol, 2002. **12**(4): p. R138-40.
8. Pasquinelli, A.E., *MicroRNAs: deviants no longer.* Trends Genet, 2002. **18**(4): p. 171-3.
9. Bhaskaran, M. and M. Mohan, *MicroRNAs: history, biogenesis, and their evolving role in animal development and disease.* Vet Pathol, 2014. **51**(4): p. 759-74.
10. Drusco, A. and C.M. Croce, *MicroRNAs and Cancer: A Long Story for Short RNAs.* Adv Cancer Res, 2017. **135**: p. 1-24.
11. Mohr, A.M. and J.L. Mott, *Overview of microRNA biology.* Semin Liver Dis, 2015. **35**(1): p. 3-11.
12. Truscott, M., A.B. Islam, and M.V. Frolov, *Novel regulation and functional interaction of polycistronic miRNAs.* RNA, 2016. **22**(1): p. 129-38.
13. Stoller, M.L., H.C. Chang, and D.M. Fekete, *Bicistronic gene transfer tools for delivery of miRNAs and protein coding sequences.* Int J Mol Sci, 2013. **14**(9): p. 18239-55.
14. Catalanotto, C., C. Cogoni, and G. Zardo, *MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions.* Int J Mol Sci, 2016. **17**(10).
15. Lee, Y., et al., *MicroRNA genes are transcribed by RNA polymerase II.* EMBO J, 2004. **23**(20): p. 4051-60.
16. Canella, D., et al., *Defining the RNA polymerase III transcriptome: Genome-wide localization of the RNA polymerase III transcription machinery in human cells.* Genome Res, 2010. **20**(6): p. 710-21.
17. Han, J., et al., *The Drosha-DGCR8 complex in primary microRNA processing.* Genes Dev, 2004. **18**(24): p. 3016-27.
18. Sperber, H., et al., *miRNA sensitivity to Drosha levels correlates with pre-miRNA secondary structure.* RNA, 2014. **20**(5): p. 621-31.
19. Yi, R., et al., *Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs.* Genes Dev, 2003. **17**(24): p. 3011-6.

20. Wu, K., et al., *The Role of Exportin-5 in MicroRNA Biogenesis and Cancer.* Genomics Proteomics Bioinformatics, 2018. **16**(2): p. 120-126.
21. Koscianska, E., J. Starega-Roslan, and W.J. Krzyzosiak, *The role of Dicer protein partners in the processing of microRNA precursors.* PLoS One, 2011. **6**(12): p. e28548.
22. Song, M.S. and J.J. Rossi, *Molecular mechanisms of Dicer: endonuclease and enzymatic activity.* Biochem J, 2017. **474**(10): p. 1603-1618.
23. Pratt, A.J. and I.J. MacRae, *The RNA-induced silencing complex: a versatile gene-silencing machine.* J Biol Chem, 2009. **284**(27): p. 17897-901.
24. Miyoshi, K., et al., *Characterization of the miRNA-RISC loading complex and miRNA-RISC formed in the Drosophila miRNA pathway.* RNA, 2009. **15**(7): p. 1282-91.
25. Kim, Y. and V.N. Kim, *MicroRNA factory: RISC assembly from precursor microRNAs.* Mol Cell, 2012. **46**(4): p. 384-6.
26. Okamura, K., N. Liu, and E.C. Lai, *Distinct mechanisms for microRNA strand selection by Drosophila Argonautes.* Mol Cell, 2009. **36**(3): p. 431-44.
27. Guo, H., et al., *Mammalian microRNAs predominantly act to decrease target mRNA levels.* Nature, 2010. **466**(7308): p. 835-40.
28. Li, M., et al., *MicroRNAs: control and loss of control in human physiology and disease.* World J Surg, 2009. **33**(4): p. 667-84.
29. Schmitz, K.M., et al., *Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes.* Genes Dev, 2010. **24**(20): p. 2264-9.
30. Nakama, M., et al., *DNA-RNA hybrid formation mediates RNAi-directed heterochromatin formation.* Genes Cells, 2012. **17**(3): p. 218-33.
31. Lu, W.T., et al., *Drosha drives the formation of DNA:RNA hybrids around DNA break sites to facilitate DNA repair.* Nat Commun, 2018. **9**(1): p. 532.
32. Li, Y., et al., *Genome-wide DNA methylome analysis reveals epigenetically dysregulated non-coding RNAs in human breast cancer.* Sci Rep, 2015. **5**: p. 8790.
33. Suzuki, H., et al., *DNA methylation and microRNA dysregulation in cancer.* Mol Oncol, 2012. **6**(6): p. 567-78.
34. van Schooneveld, E., et al., *Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management.* Breast Cancer Res, 2015. **17**: p. 21.
35. Vrba, L., et al., *miRNA gene promoters are frequent targets of aberrant DNA methylation in human breast cancer.* PLoS One, 2013. **8**(1): p. e54398.
36. Wang, S., W. Wu, and F.X. Claret, *Mutual regulation of microRNAs and DNA methylation in human cancers.* Epigenetics, 2017. **12**(3): p. 187-197.
37. Cortese, F., et al., *Vive la radioresistance!: converging research in radiobiology and biogerontology to enhance human radioresistance for deep space exploration and colonization.* Oncotarget, 2018. **9**(18): p. 14692-14722.
38. Cucinotta, F.A., K. To, and E. Cacao, *Predictions of space radiation fatality risk for exploration missions.* Life Sci Space Res (Amst), 2017. **13**: p. 1-11.

39. Durante, M. and F.A. Cucinotta, *Heavy ion carcinogenesis and human space exploration.* Nat Rev Cancer, 2008. **8**(6): p. 465-72.
40. Alpen, E.L., et al., *Fluence-based relative biological effectiveness for charged particle carcinogenesis in mouse Harderian gland.* Adv Space Res, 1994. **14**(10): p. 573-81.
41. Beheshti, A., et al., *NASA GeneLab Project: Bridging Space Radiation Omics with Ground Studies.* Radiat Res, 2018. **189**(6): p. 553-559.
42. Chancellor, J.C., G.B. Scott, and J.P. Sutton, *Space Radiation: The Number One Risk to Astronaut Health beyond Low Earth Orbit.* Life (Basel), 2014. **4**(3): p. 491-510.
43. Blaber, E., H. Marcal, and B.P. Burns, *Bioastronautics: the influence of microgravity on astronaut health.* Astrobiology, 2010. **10**(5): p. 463-73.
44. Moreno-Villanueva, M., et al., *Interplay of space radiation and microgravity in DNA damage and DNA damage response.* NPJ Microgravity, 2017. **3**: p. 14.
45. Parra, M., et al., *Microgravity validation of a novel system for RNA isolation and multiplex quantitative real time PCR analysis of gene expression on the International Space Station.* PLoS One, 2017. **12**(9): p. e0183480.
46. Teodori, L., et al., *Skeletal Muscle Atrophy in Simulated Microgravity Might Be Triggered by Immune-Related microRNAs.* Front Physiol, 2019. **9**: p. 1926.
47. Girardi, C., et al., *Analysis of miRNA and mRNA Expression Profiles Highlights Alterations in Ionizing Radiation Response of Human Lymphocytes under Modeled Microgravity.* PLoS ONE, 2012. **7**(2): p. e31293.
48. Xie, Y. and Y. Chen, *microRNAs: Emerging Targets Regulating Oxidative Stress in the Models of Parkinson's Disease.* Front Neurosci, 2016. **10**: p. 298.
49. Gao, Y., et al., *Changes in apoptotic microRNA and mRNA expression profiling in Caenorhabditis elegans during the Shenzhou-8 mission.* J Radiat Res, 2015. **56**(6): p. 872-82.
50. Czochor, J.R. and P.M. Glazer, *microRNAs in cancer cell response to ionizing radiation.* Antioxid Redox Signal, 2014. **21**(2): p. 293-312.
51. Fendler, W., et al., *Evolutionarily conserved serum microRNAs predict radiation-induced fatality in nonhuman primates.* Sci Transl Med, 2017. **9**(379).
52. Beheshti, A., et al., *GeneLab Database Analyses Suggest Long-Term Impact of Space Radiation on the Cardiovascular System by the Activation of FYN Through Reactive Oxygen Species.* Int J Mol Sci, 2019. **20**(3).
53. Qin, S.B., et al., *MiR-182-5p Inhibited Oxidative Stress and Apoptosis Triggered by Oxidized Low-Density Lipoprotein via Targeting Toll-Like Receptor 4.* J Cell Physiol, 2017.
54. Wright, C.M., et al., *microRNAs: The Short Link between Cancer and RT-Induced DNA Damage Response.* Front Oncol, 2014. **4**: p. 133.
55. Allen, D.L., et al., *Effects of spaceflight on murine skeletal muscle gene expression.* J Appl Physiol (1985), 2009. **106**(2): p. 582-95.
56. Girardi, C., et al., *Integration analysis of microRNA and mRNA expression profiles in human peripheral blood lymphocytes cultured in modeled microgravity.* Biomed Res Int, 2014. **2014**: p. 296747.

57. Girardi, C., et al., *Analysis of miRNA and mRNA expression profiles highlights alterations in ionizing radiation response of human lymphocytes under modeled microgravity.* PLoS One, 2012. **7**(2): p. e31293.
58. Hughes-Fulford, M., et al., *Spaceflight alters expression of microRNA during T-cell activation.* FASEB J, 2015. **29**(12): p. 4893-900.
59. Mangala, L.S., et al., *Effects of simulated microgravity on expression profile of microRNA in human lymphoblastoid cells.* J Biol Chem, 2011. **286**(37): p. 32483-90.
60. Ling, S., et al., *Circulating microRNAs Correlated with Bone Loss Induced by 45 Days of Bed Rest.* Front Physiol, 2017. **8**: p. 69.
61. Metheetrairut, C. and F.J. Slack, *MicroRNAs in the ionizing radiation response and in radiotherapy.* Curr Opin Genet Dev, 2013. **23**(1): p. 12-9.
62. Aquino-Jarquin, G., *Emerging Role of CRISPR/Cas9 Technology for MicroRNAs Editing in Cancer Research.* Cancer Res, 2017. **77**(24): p. 6812-6817.
63. Beheshti, A., et al., *A microRNA signature and TGF-beta1 response were identified as the key master regulators for spaceflight response.* PLoS One, 2018. **13**(7): p. e0199621.
64. Ray, S., et al., *GeneLab: Omics database for spaceflight experiments.* Bioinformatics, 2018.
65. Inamura, K., *Major Tumor Suppressor and Oncogenic Non-Coding RNAs: Clinical Relevance in Lung Cancer.* Cells, 2017. **6**(2).
66. Wu, L., et al., *Precise let-7 expression levels balance organ regeneration against tumor suppression.* Elife, 2015. **4**: p. e09431.
67. Lehmann, S.M., et al., *An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration.* Nat Neurosci, 2012. **15**(6): p. 827-35.
68. Kumarswamy, R., I. Volkmann, and T. Thum, *Regulation and function of miRNA-21 in health and disease.* RNA Biol, 2011. **8**(5): p. 706-13.
69. Choudhury, S.N. and Y. Li, *miR-21 and let-7 in the Ras and NF-kappaB pathways.* Microrna, 2012. **1**(1): p. 65-9.
70. Shishodia, G., et al., *Alterations in microRNAs miR-21 and let-7a correlate with aberrant STAT3 signaling and downstream effects during cervical carcinogenesis.* Mol Cancer, 2015. **14**: p. 116.
71. Morgoulis, D., et al., *sPIF promotes myoblast differentiation and utrophin expression while inhibiting fibrosis in Duchenne muscular dystrophy via the H19/miR-675/let-7 and miR-21 pathways.* Cell Death Dis, 2019. **10**(2): p. 82.
72. Beheshti, A., et al., *A Circulating microRNA Signature Predicts Age-Based Development of Lymphoma.* PLoS One, 2017. **12**(1): p. e0170521.
73. Berezikov, E., *Evolution of microRNA diversity and regulation in animals.* Nat Rev Genet, 2011. **12**(12): p. 846-60.
74. Moran, Y., et al., *The evolutionary origin of plant and animal microRNAs.* Nat Ecol Evol, 2017. **1**(3): p. 27.
75. Li, Z., R. Xu, and N. Li, *MicroRNAs from plants to animals, do they define a new messenger for communication?* Nutr Metab (Lond), 2018. **15**: p. 68.

76. Alwood, J.S., et al., *From the bench to exploration medicine: NASA life sciences translational research for human exploration and habitation missions.* NPJ Microgravity, 2017. **3**: p. 5.
77. Aceto, J., et al., *Zebrafish Bone and General Physiology Are Differently Affected by Hormones or Changes in Gravity.* PLoS One, 2015. **10**(6): p. e0126928.
78. Aceto, J., et al., *Effects of microgravity simulation on zebrafish transcriptomes and bone physiology-exposure starting at 5 days post fertilization.* NPJ Microgravity, 2016. **2**: p. 16010.
79. Chatani, M., et al., *Acute transcriptional up-regulation specific to osteoblasts/osteoclasts in medaka fish immediately after exposure to microgravity.* Sci Rep, 2016. **6**: p. 39545.
80. Murata, Y., et al., *Histological and Transcriptomic Analysis of Adult Japanese Medaka Sampled Onboard the International Space Station.* PLoS One, 2015. **10**(10): p. e0138799.
81. Chang, T.C. and J.T. Mendell, *microRNAs in vertebrate physiology and human disease.* Annu Rev Genomics Hum Genet, 2007. **8**: p. 215-39.
82. Li, G., et al., *miRNA targeted signaling pathway in the early stage of denervated fast and slow muscle atrophy.* Neural Regen Res, 2016. **11**(8): p. 1293-303.
83. Teodori, L., et al., *Skeletal Muscle Atrophy in Simulated Microgravity Might Be Triggered by Immune-Related microRNAs.* Front Physiol, 2018. **9**: p. 1926.
84. Rullman, E., et al., *PlanHab (Planetary Habitat Simulation): the combined and separate effects of 21 days bed rest and hypoxic confinement on human skeletal muscle miRNA expression.* Physiol Rep, 2016. **4**(8).
85. Baio, J., et al., *Cardiovascular progenitor cells cultured aboard the International Space Station exhibit altered developmental and functional properties.* NPJ Microgravity, 2018. **4**: p. 13.
86. Cui, Y., et al., *Systematic Analysis of mRNA and miRNA Expression of 3D-Cultured Neural Stem Cells (NSCs) in Spaceflight.* Front Cell Neurosci, 2017. **11**: p. 434.
87. Xu, D., et al., *Changes in miRNA expression profile of space-flown Caenorhabditis elegans during Shenzhou-8 mission.* Life Sci Space Res (Amst), 2014. **1**: p. 44-52.
88. Higashitani, A., et al., *C. elegans RNAi space experiment (CERISE) in Japanese Experiment Module KIBO.* Biol Sci Space, 2009. **23**(4): p. 183-187.
89. Higashibata, A., et al., *Microgravity elicits reproducible alterations in cytoskeletal and metabolic gene and protein expression in space-flown Caenorhabditis elegans.* NPJ Microgravity, 2016. **2**: p. 15022.
90. Etheridge, T., et al., *The effectiveness of RNAi in Caenorhabditis elegans is maintained during spaceflight.* PLoS One, 2011. **6**(6): p. e20459.
91. Adenle, A.A., B. Johnsen, and N.J. Szewczyk, *Review of the results from the International C. elegans first experiment (ICE-FIRST).* Adv Space Res, 2009. **44**(2): p. 210-216.
92. Honda, Y., et al., *Genes down-regulated in spaceflight are involved in the control of longevity in Caenorhabditis elegans.* Sci Rep, 2012. **2**: p. 487.
93. Hateley, S., et al., *Transcriptomic response of Drosophila melanogaster pupae developed in hypergravity.* Genomics, 2016. **108**(3-4): p. 158-167.
94. Marcu, O., et al., *Innate immune responses of Drosophila melanogaster are altered by spaceflight.* PLoS One, 2011. **6**(1): p. e15361.

95. Taylor, K., et al., *Toll mediated infection response is altered by gravity and spaceflight in Drosophila.* PLoS One, 2014. **9**(1): p. e86485.

96. Ogneva, I.V., S.N. Belyakin, and S.V. Sarantseva, *The Development Of Drosophila Melanogaster under Different Duration Space Flight and Subsequent Adaptation to Earth Gravity.* PLoS One, 2016. **11**(11): p. e0166885.

97. Hosamani, R., et al., *Elucidating the "Gravome": Quantitative Proteomic Profiling of the Response to Chronic Hypergravity in Drosophila.* J Proteome Res, 2016. **15**(12): p. 4165-4175.

98. Basu, P., et al., *Growth in spaceflight hardware results in alterations to the transcriptome and proteome.* Life Sci Space Res (Amst), 2017. **15**: p. 88-96.

99. Basu, P., D.R. Luesse, and S.E. Wyatt, *Proteomic approaches and their application to plant gravitropism.* Methods Mol Biol, 2015. **1309**: p. 119-32.

100. Choi, W.G., et al., *Variation in the transcriptome of different ecotypes of Arabidopsis thaliana reveals signatures of oxidative stress in plant responses to spaceflight.* Am J Bot, 2019. **106**(1): p. 123-136.

101. Ferl, R.J., et al., *Spaceflight induces specific alterations in the proteomes of Arabidopsis.* Astrobiology, 2015. **15**(1): p. 32-56.

102. Kruse, C.P.S., et al., *Transcriptome and proteome responses in RNAlater preserved tissue of Arabidopsis thaliana.* PLoS One, 2017. **12**(4): p. e0175943.

103. Paul, A.L., et al., *Organ-specific remodeling of the Arabidopsis transcriptome in response to spaceflight.* BMC Plant Biol, 2013. **13**: p. 112.

104. Visscher, A.M., et al., *Growth performance and root transcriptome remodeling of Arabidopsis in response to Mars-like levels of magnesium sulfate.* PLoS One, 2010. **5**(8): p. e12348.

105. Vandenbrink, J.P. and J.Z. Kiss, *Space, the final frontier: A critical review of recent experiments performed in microgravity.* Plant Sci, 2016. **243**: p. 115-9.

106. Woodward, A.W. and B. Bartel, *Biology in Bloom: A Primer on the Arabidopsis thaliana Model System.* Genetics, 2018. **208**(4): p. 1337-1349.

107. Llave, C., et al., *Endogenous and silencing-associated small RNAs in plants.* Plant Cell, 2002. **14**(7): p. 1605-19.

108. Park, W., et al., *CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in Arabidopsis thaliana.* Curr Biol, 2002. **12**(17): p. 1484-95.

109. Reinhart, B.J., et al., *MicroRNAs in plants.* Genes Dev, 2002. **16**(13): p. 1616-26.

110. Voinnet, O., *Origin, biogenesis, and activity of plant microRNAs.* Cell, 2009. **136**(4): p. 669-87.

111. Zhang, B., et al., *Conservation and divergence of plant microRNA genes.* Plant J, 2006. **46**(2): p. 243-59.

112. Xu, D., S. Guo, and M. Liu, *Identification of miRNAs involved in long-term simulated microgravity response in Solanum lycopersicum.* Plant Physiol Biochem, 2013. **66**: p. 10-9.

113. Checinska Sielaff, A., et al., *Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces.* Microbiome, 2019. **7**(1): p. 50.

114. Singh, N.K., et al., *Draft Genome Sequences of Several Fungal Strains Selected for Exposure to Microgravity at the International Space Station.* Genome Announc, 2017. **5**(15).

115. Haines, S.R., et al., *Quantitative evaluation of bioaerosols in different particle size fractions in dust collected on the International Space Station (ISS).* Appl Microbiol Biotechnol, 2019. **103**(18): p. 7767-7782.

116. Urbaniak, C., et al., *Detection of antimicrobial resistance genes associated with the International Space Station environmental surfaces.* Sci Rep, 2018. **8**(1): p. 814.

117. Morrison, M.D., P. Fajardo-Cavazos, and W.L. Nicholson, *Comparison of Bacillus subtilis transcriptome profiles from two separate missions to the International Space Station.* NPJ Microgravity, 2019. **5**: p. 1.

118. Morrison, M.D. and W.L. Nicholson, *Meta-analysis of data from spaceflight transcriptome experiments does not support the idea of a common bacterial "spaceflight response".* Sci Rep, 2018. **8**(1): p. 14403.

119. Crabbe, A., et al., *Transcriptional and proteomic responses of Pseudomonas aeruginosa PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen.* Appl Environ Microbiol, 2011. **77**(4): p. 1221-30.

120. Urbaniak, C. and G. Reid, *The potential influence of the microbiota and probiotics on women during long spaceflights.* Womens Health (Lond), 2016. **12**(2): p. 193-8.

121. Sakai, T., et al., *Probiotics into outer space: feasibility assessments of encapsulated freeze-dried probiotics during 1 month's storage on the International Space Station.* Sci Rep, 2018. **8**(1): p. 10687.

122. Voorhies, A.A. and H.A. Lorenzi, *The Challenge of Maintaining a Healthy Microbiome during Long-Duration Space Missions.* Frontiers in Astronomy and Space Sciences, 2016. **3**(23).

123. Crucian, B.E., et al., *Immune System Dysregulation During Spaceflight: Potential Countermeasures for Deep Space Exploration Missions.* Front Immunol, 2018. **9**: p. 1437.

124. Nickerson, C.A., et al., *Microbial responses to microgravity and other low-shear environments.* Microbiol Mol Biol Rev, 2004. **68**(2): p. 345-61.

125. Quigley, E.M., *Gut bacteria in health and disease.* Gastroenterol Hepatol (N Y), 2013. **9**(9): p. 560-9.

126. Liu, S., et al., *The Host Shapes the Gut Microbiota via Fecal MicroRNA.* Cell Host Microbe, 2016. **19**(1): p. 32-43.

127. Yuan, C., et al., *Interaction between Host MicroRNAs and the Gut Microbiota in Colorectal Cancer.* mSystems, 2018. **3**(3).

128. David, L.A., et al., *Diet rapidly and reproducibly alters the human gut microbiome.* Nature, 2014. **505**(7484): p. 559-63.

129. Daien, C.I., et al., *Detrimental Impact of Microbiota-Accessible Carbohydrate-Deprived Diet on Gut and Immune Homeostasis: An Overview.* Front Immunol, 2017. **8**: p. 548.

130. Zeng, H., D.L. Lazarova, and M. Bordonaro, *Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention.* World J Gastrointest Oncol, 2014. **6**(2): p. 41-51.

131. Hu, S., et al., *Butyrate inhibits pro-proliferative miR-92a by diminishing c-Myc-induced miR-17-92a cluster transcription in human colon cancer cells.* Mol Cancer, 2015. **14**: p. 180.

132. Rodriguez-Nogales, A., et al., *Differential intestinal anti-inflammatory effects of Lactobacillus fermentum and Lactobacillus salivarius in DSS mouse colitis: impact on microRNAs expression and microbiota composition.* Mol Nutr Food Res, 2017. **61**(11).

133. Rodriguez-Nogales, A., et al., *Intestinal anti-inflammatory effect of the probiotic Saccharomyces boulardii in DSS-induced colitis in mice: Impact on microRNAs expression and gut microbiota composition.* J Nutr Biochem, 2018. **61**: p. 129-139.

134. Kumagai, T., F. Rahman, and A.M. Smith, *The Microbiome and Radiation Induced-Bowel Injury: Evidence for Potential Mechanistic Role in Disease Pathogenesis.* Nutrients, 2018. **10**(10).

135. Lacey, J.A., et al., *Clostridium perfringens-mediated necrotic enteritis is not influenced by the pre-existing microbiota but is promoted by large changes in the post-challenge microbiota.* Vet Microbiol, 2018. **227**: p. 119-126.

136. Shimizu, K., et al., *Synbiotics modulate gut microbiota and reduce enteritis and ventilator-associated pneumonia in patients with sepsis: a randomized controlled trial.* Crit Care, 2018. **22**(1): p. 239.

137. Rooney, B.V., et al., *Herpes Virus Reactivation in Astronauts During Spaceflight and Its Application on Earth.* Front Microbiol, 2019. **10**: p. 16.

138. Piedade, D. and J.M. Azevedo-Pereira, *The Role of microRNAs in the Pathogenesis of Herpesvirus Infection.* Viruses, 2016. **8**(6).

139. Bennasser, Y., M.L. Yeung, and K.T. Jeang, *HIV-1 TAR RNA subverts RNA interference in transfected cells through sequestration of TAR RNA-binding protein, TRBP.* J Biol Chem, 2006. **281**(38): p. 27674-8.

140. Bruscella, P., et al., *Viruses and miRNAs: More Friends than Foes.* Front Microbiol, 2017. **8**: p. 824.

141. Cullen, B.R., *How do viruses avoid inhibition by endogenous cellular microRNAs?* PLoS Pathog, 2013. **9**(11): p. e1003694.

142. Luna, J.M., et al., *Hepatitis C virus RNA functionally sequesters miR-122.* Cell, 2015. **160**(6): p. 1099-110.

143. Grundhoff, A. and C.S. Sullivan, *Virus-encoded microRNAs.* Virology, 2011. **411**(2): p. 325-43.

144. Harwig, A., A.T. Das, and B. Berkhout, *Retroviral microRNAs.* Curr Opin Virol, 2014. **7**: p. 47-54.

145. Cokaric Brdovcak, M., A. Zubkovic, and I. Jurak, *Herpes Simplex Virus 1 Deregulation of Host MicroRNAs.* Noncoding RNA, 2018. **4**(4).

146. Weber, J.A., et al., *The microRNA spectrum in 12 body fluids.* Clin Chem, 2010. **56**(11): p. 1733-41.

147. Silva, S.S., et al., *Forensic miRNA: potential biomarker for body fluids?* Forensic Sci Int Genet, 2015. **14**: p. 1-10.

148. Glinge, C., et al., *Stability of Circulating Blood-Based MicroRNAs - Pre-Analytic Methodological Considerations.* PLoS One, 2017. **12**(2): p. e0167969.

149. Terrinoni, A., et al., *The circulating miRNAs as diagnostic and prognostic markers.* Clin Chem Lab Med, 2019. **57**(7): p. 932-953.

150. Zhu, H. and G.C. Fan, *Extracellular/circulating microRNAs and their potential role in cardiovascular disease.* Am J Cardiovasc Dis, 2011. **1**(2): p. 138-149.

151. Shigeyasu, K., et al., *Emerging Role of MicroRNAs as Liquid Biopsy Biomarkers in Gastrointestinal Cancers.* Clin Cancer Res, 2017. **23**(10): p. 2391-2399.

152. Cortez, M.A., et al., *MicroRNAs in body fluids--the mix of hormones and biomarkers.* Nat Rev Clin Oncol, 2011. **8**(8): p. 467-77.

153. Vickers, K.C., et al., *MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins.* Nat Cell Biol, 2011. **13**(4): p. 423-33.

154. Faraldi, M., et al., *Normalization strategies differently affect circulating miRNA profile associated with the training status.* Sci Rep, 2019. **9**(1): p. 1584.

155. Kawaguchi, T., et al., *Circulating MicroRNAs: A Next-Generation Clinical Biomarker for Digestive System Cancers.* Int J Mol Sci, 2016. **17**(9).

156. Uratani, R., et al., *Diagnostic Potential of Cell-Free and Exosomal MicroRNAs in the Identification of Patients with High-Risk Colorectal Adenomas.* PLoS One, 2016. **11**(10): p. e0160722.

157. Jayaram, H., et al., *MicroRNA Expression in the Glaucomatous Retina.* Invest Ophthalmol Vis Sci, 2015. **56**(13): p. 7971-82.

158. Joglekar, M.V., et al., *Circulating microRNA Biomarkers of Diabetic Retinopathy.* Diabetes, 2016. **65**(1): p. 22-4.

159. Raghunath, A. and E. Perumal, *Micro-RNAs and their roles in eye disorders.* Ophthalmic Res, 2015. **53**(4): p. 169-86.

160. Acharya, S.S., et al., *Serum microRNAs are early indicators of survival after radiation-induced hematopoietic injury.* Sci Transl Med, 2015. **7**(287): p. 287ra69.

161. Zampetaki, A. and M. Mayr, *Circulating microRNAs as Novel Biomarkers in Cardiovascular Disease: Basic and Technical Principles*, in *Non-coding RNAs in the Vasculature*, T. Thum and S. Dimmeler, Editors. 2017, Springer International Publishing: Cham. p. 83-101.

162. Jansen, F., et al., *Kinetics of Circulating MicroRNAs in Response to Cardiac Stress in Patients With Coronary Artery Disease.* J Am Heart Assoc, 2017. **6**(8).

163. Viereck, J. and T. Thum, *Circulating Noncoding RNAs as Biomarkers of Cardiovascular Disease and Injury.* Circ Res, 2017. **120**(2): p. 381-399.

164. Coenen-Stass, A.M.L., M.J.A. Wood, and T.C. Roberts, *Biomarker Potential of Extracellular miRNAs in Duchenne Muscular Dystrophy.* Trends Mol Med, 2017. **23**(11): p. 989-1001.

165. Li, X., et al., *Circulating Muscle-specific miRNAs in Duchenne Muscular Dystrophy Patients.* Mol Ther Nucleic Acids, 2014. **3**: p. e177.

166. Tasca, E., et al., *Circulating microRNAs as biomarkers of muscle differentiation and atrophy in ALS.* Clin Neuropathol, 2016. **35**(1): p. 22-30.

167. Summerer, I., et al., *Changes in circulating microRNAs after radiochemotherapy in head and neck cancer patients.* Radiat Oncol, 2013. **8**: p. 296.

168. Wozniak, M.B., et al., *Circulating MicroRNAs as Non-Invasive Biomarkers for Early Detection of Non-Small-Cell Lung Cancer.* PLoS One, 2015. **10**(5): p. e0125026.

169. Wang, W.T. and Y.Q. Chen, *Circulating miRNAs in cancer: from detection to therapy.* J Hematol Oncol, 2014. **7**: p. 86.

170. Koturbash, I., et al., *Sex-specific microRNAome deregulation in the shielded bystander spleen of cranially exposed mice.* Cell Cycle, 2008. **7**(11): p. 1658-67.

171. Ronca, A.E., et al., *Effects of sex and gender on adaptations to space: reproductive health.* J Womens Health (Larchmt), 2014. **23**(11): p. 967-74.

172. Mark, S., et al., *The impact of sex and gender on adaptation to space: executive summary.* J Womens Health (Larchmt), 2014. **23**(11): p. 941-7.

173. Kennedy, A.R., et al., *Effects of sex and gender on adaptation to space: immune system.* J Womens Health (Larchmt), 2014. **23**(11): p. 956-8.

174. Koturbash, I., et al., *Sex-specific radiation-induced microRNAome responses in the hippocampus, cerebellum and frontal cortex in a mouse model.* Mutat Res, 2011. **722**(2): p. 114-8.

175. Sachs, R.K., L.R. Hlatky, and B.J. Trask, *Radiation-produced chromosome aberrations: colourful clues.* Trends Genet, 2000. **16**(4): p. 143-6.

176. Hahnfeldt, P., et al., *Chromosome aberrations produced by radiation: the relationship between excess acentric fragments and dicentrics.* Radiat Res, 1995. **141**(2): p. 136-52.

177. Hlatky, L., et al., *Radiation-induced chromosome aberrations: insights gained from biophysical modeling.* Bioessays, 2002. **24**(8): p. 714-23.

178. Durante, M. and S.C. Formenti, *Radiation-Induced Chromosomal Aberrations and Immunotherapy: Micronuclei, Cytosolic DNA, and Interferon-Production Pathway.* Front Oncol, 2018. **8**: p. 192.

179. McMahon, S.J. and K.M. Prise, *Mechanistic Modelling of Radiation Responses.* Cancers (Basel), 2019. **11**(2).

180. Calin, G.A. and C.M. Croce, *MicroRNAs and chromosomal abnormalities in cancer cells.* Oncogene, 2006. **25**(46): p. 6202-10.

181. Calin, G.A. and C.M. Croce, *Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications.* J Clin Invest, 2007. **117**(8): p. 2059-66.

182. Calin, G.A., et al., *Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers.* Proc Natl Acad Sci U S A, 2004. **101**(9): p. 2999-3004.

183. Calin, G.A., et al., *Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia.* Proc Natl Acad Sci U S A, 2002. **99**(24): p. 15524-9.

184. Sonoki, T., et al., *Insertion of microRNA-125b-1, a human homologue of lin-4, into a rearranged immunoglobulin heavy chain gene locus in a patient with precursor B-cell acute lymphoblastic leukemia.* Leukemia, 2005. **19**(11): p. 2009-10.

185. Liamina, D., et al., *Radiation-Induced Changes of microRNA Expression Profiles in Radiosensitive and Radioresistant Leukemia Cell Lines with Different Levels of Chromosome Abnormalities.* Cancers (Basel), 2017. **9**(10).

186. Lages, E., et al., *MicroRNAs: molecular features and role in cancer.* Front Biosci (Landmark Ed), 2012. **17**: p. 2508-40.

187. Surova, O., N.S. Akbar, and B. Zhivotovsky, *Knock-down of core proteins regulating microRNA biogenesis has no effect on sensitivity of lung cancer cells to ionizing radiation.* PLoS One, 2012. **7**(3): p. e33134.

188. Tomasik, B., W. Fendler, and D. Chowdhury, *Serum microRNAs - potent biomarkers for radiation biodosimetry.* Oncotarget, 2018. **9**(18): p. 14038-14039.

189. Cui, W., et al., *Plasma miRNA as biomarkers for assessment of total-body radiation exposure dosimetry.* PLoS One, 2011. **6**(8): p. e22988.

190. Templin, T., et al., *Radiation-induced micro-RNA expression changes in peripheral blood cells of radiotherapy patients.* Int J Radiat Oncol Biol Phys, 2011. **80**(2): p. 549-57.

191. Singh, V.K. and H.B. Pollard, *Ionizing radiation-induced altered microRNA expression as biomarkers for assessing acute radiation injury.* Expert Rev Mol Diagn, 2017. **17**(10): p. 871-874.

192. Li, X.H., et al., *Delta-tocotrienol suppresses radiation-induced microRNA-30 and protects mice and human CD34+ cells from radiation injury.* PLoS One, 2015. **10**(3): p. e0122258.

193. Jacob, N.K., et al., *Identification of sensitive serum microRNA biomarkers for radiation biodosimetry.* PLoS One, 2013. **8**(2): p. e57603.

194. Ahmad, P., et al., *MicroRNA-15b-5p Predicts Locoregional Relapse in Head and Neck Carcinoma Patients Treated With Intensity-modulated Radiotherapy.* Cancer Genomics Proteomics, 2019. **16**(2): p. 139-146.

195. Khan, S.Y., et al., *Distinctive microRNA expression signatures in proton-irradiated mice.* Mol Cell Biochem, 2013. **382**(1-2): p. 225-35.

196. Templin, T., et al., *Whole mouse blood microRNA as biomarkers for exposure to gamma-rays and (56)Fe ion.* Int J Radiat Biol, 2011. **87**(7): p. 653-62.

197. Zhang, Y., et al., *Transient gene and microRNA expression profile changes of confluent human fibroblast cells in spaceflight.* FASEB J, 2016. **30**(6): p. 2211-24.

198. Wei, W., et al., *Serum microRNA as noninvasive indicator for space radiation.* Acta Astronautica, 2018. **152**: p. 101-104.

199. Wang, K., et al., *Circulating microRNAs, potential biomarkers for drug-induced liver injury.* Proc Natl Acad Sci U S A, 2009. **106**(11): p. 4402-7.

200. Murach, K.A. and J.J. McCarthy, *MicroRNAs, heart failure, and aging: potential interactions with skeletal muscle.* Heart Fail Rev, 2017. **22**(2): p. 209-218.

201. Cheng, M., et al., *Circulating myocardial microRNAs from infarcted hearts are carried in exosomes and mobilise bone marrow progenitor cells.* Nat Commun, 2019. **10**(1): p. 959.

202. Mas, V.R., et al., *MicroRNAs as biomarkers in solid organ transplantation.* Am J Transplant, 2013. **13**(1): p. 11-9.

203. Shapiro, M.D., et al., *MicroRNA expression data reveals a signature of kidney damage following ischemia reperfusion injury.* PLoS One, 2011. **6**(8): p. e23011.

204. Sui, W., et al., *Microarray analysis of MicroRNA expression in acute rejection after renal transplantation.* Transpl Immunol, 2008. **19**(1): p. 81-5.

205. Anglicheau, D., et al., *MicroRNA expression profiles predictive of human renal allograft status.* Proc Natl Acad Sci U S A, 2009. **106**(13): p. 5330-5.

206. Scian, M.J., et al., *MicroRNA profiles in allograft tissues and paired urines associate with chronic allograft dysfunction with IF/TA.* Am J Transplant, 2011. **11**(10): p. 2110-22.

207. Hummel, R., D.J. Hussey, and J. Haier, *MicroRNAs: predictors and modifiers of chemo- and radiotherapy in different tumour types.* Eur J Cancer, 2010. **46**(2): p. 298-311.

208. Menon, N., et al., *Detection of Acute Radiation Sickness: A Feasibility Study in Non-Human Primates Circulating miRNAs for Triage in Radiological Events.* PLoS One, 2016. **11**(12): p. e0167333.

209. Globus, R.K. and E. Morey-Holton, *Hindlimb unloading: rodent analog for microgravity.* J Appl Physiol (1985), 2016. **120**(10): p. 1196-206.

210. Tahimic, C.G.T., et al., *Influence of Social Isolation During Prolonged Simulated Weightlessness by Hindlimb Unloading.* Front Physiol, 2019. **10**: p. 1147.

211. Ikeda, H., et al., *Development and performance evaluation of a three-dimensional clinostat synchronized heavy-ion irradiation system.* Life Sci Space Res (Amst), 2017. **12**: p. 51-60.

212. Ikeda, H., et al., *Expression Profile of Cell Cycle-Related Genes in Human Fibroblasts Exposed Simultaneously to Radiation and Simulated Microgravity.* Int J Mol Sci, 2019. **20**(19).

213. Tackett, N., et al., *Prolonged exposure to simulated microgravity diminishes dendritic cell immunogenicity.* Sci Rep, 2019. **9**(1): p. 13825.

214. Huyan, T., et al., *Evaluation of osteoclast-derived exosomal miRNA under simulated microgravity conditions using next-generation sequencing.* Acta Astronautica, 2019. **161**: p. 75-86.

215. Qin, W., et al., *Mir-494 inhibits osteoblast differentiation by regulating BMP signaling in simulated microgravity.* Endocrine, 2019. **65**(2): p. 426-439.

216. Sun, Z., et al., *miR-181c-5p mediates simulated microgravity-induced impaired osteoblast proliferation by promoting cell cycle arrested in the G2 phase.* J Cell Mol Med, 2019. **23**(5): p. 3302-3316.

217. Hu, Z., et al., *miRNA-132-3p inhibits osteoblast differentiation by targeting Ep300 in simulated microgravity.* Sci Rep, 2015. **5**: p. 18655.

218. Xu, H., et al., *Effects of simulated microgravity on microRNA and mRNA expression profile of rat soleus.* Acta Astronautica, 2015. **107**: p. 40-49.

219. Girardi, C., et al., *Integration Analysis of MicroRNA and mRNA Expression Profiles in Human Peripheral Blood Lymphocytes Cultured in Modeled Microgravity.* BioMed Research International, 2014. **2014**: p. 16.

220. Ade, C.J. and D.A. Bemben, *Differential MicroRNA expression following head-down tilt bed rest: implications for cardiovascular responses to microgravity.* Physiol Rep, 2019. **7**(9): p. e14061.

221. Crucian, B.E., et al., *Immune system dysregulation following short-vs long-duration spaceflight.* Aviation, space, and environmental medicine, 2008. **79**(9): p. 835-843.

222. Stowe, R.P., C.F. Sams, and D.L. Pierson, *Effects of mission duration on neuroimmune responses in astronauts.* Aviat Space Environ Med, 2003. **74**(12): p. 1281-4.

223. Stowe, R.P., C.F. Sams, and D.L. Pierson, *Adrenocortical and immune responses following short- and long-duration spaceflight.* Aviat Space Environ Med, 2011. **82**(6): p. 627-34.

224. Konstantinova, I., et al., *Cellular and humoral immunity in cosmonauts as affected by space flight factors.* Vestnik Akademii meditsinskikh nauk SSSR, 1985(8): p. 51-58.

225. Kaur, I., et al., *Changes in neutrophil functions in astronauts.* Brain, behavior, and immunity, 2004. **18**(5): p. 443-450.

226. Stowe, R.P., C.F. Sams, and D.L. Pierson, *Effects of mission duration on neuroimmune responses in astronauts.* Aviation, space, and environmental medicine, 2003. **74**(12): p. 1281-1284.

227. D'Mello, C. and M.G. Swain, *Liver-brain inflammation axis.* Am J Physiol Gastrointest Liver Physiol, 2011. **301**(5): p. G749-61.

228. D'Mello, C. and M.G. Swain, *Liver-brain interactions in inflammatory liver diseases: implications for fatigue and mood disorders.* Brain Behav Immun, 2014. **35**: p. 9-20.

229. Wu, J.Y., et al., *Osteoblastic regulation of B lymphopoiesis is mediated by Gs{alpha}-dependent signaling pathways.* Proc Natl Acad Sci U S A, 2008. **105**(44): p. 16976-81.

230. Panaroni, C., et al., *PTH Signaling in Osteoprogenitors Is Essential for B-Lymphocyte Differentiation and Mobilization.* J Bone Miner Res, 2015. **30**(12): p. 2273-86.

231. Green, A.C., et al., *Mesenchymal lineage cells and their importance in B lymphocyte niches.* Bone, 2019. **119**: p. 42-56.

232. Valderrabano, R.J., et al., *Older Men With Anemia Have Increased Fracture Risk Independent of Bone Mineral Density.* J Clin Endocrinol Metab, 2017. **102**(7): p. 2199-2206.

233. Orwoll, E.S., et al., *Skeletal health in long-duration astronauts: nature, assessment, and management recommendations from the NASA Bone Summit.* J Bone Miner Res, 2013. **28**(6): p. 1243-55.

234. Vico, L., et al., *Cortical and Trabecular Bone Microstructure Did Not Recover at Weight-Bearing Skeletal Sites and Progressively Deteriorated at Non-Weight-Bearing Sites During the Year Following International Space Station Missions.* J Bone Miner Res, 2017. **32**(10): p. 2010-2021.

235. Nishida, K. and K. Otsu, *Inflammation and metabolic cardiomyopathy.* Cardiovasc Res, 2017. **113**(4): p. 389-398.

236. Kawai, C., *From myocarditis to cardiomyopathy: mechanisms of inflammation and cell death: learning from the past for the future.* Circulation, 1999. **99**(8): p. 1091-1100.

237. Ryan, B.M., A.I. Robles, and C.C. Harris, *Genetic variation in microRNA networks: the implications for cancer research.* Nat Rev Cancer, 2010. **10**(6): p. 389-402.

238. Mehta, A. and D. Baltimore, *MicroRNAs as regulatory elements in immune system logic.* Nature Reviews Immunology, 2016. **16**(5): p. 279.

239. Mehta, A. and D. Baltimore, *MicroRNAs as regulatory elements in immune system logic.* Nat Rev Immunol, 2016. **16**(5): p. 279-94.

240. Gracias, D.T. and P.D. Katsikis, *MicroRNAs: key components of immune regulation.* Adv Exp Med Biol, 2011. **780**: p. 15-26.

241. Nejad, C., H.J. Stunden, and M.P. Gantier, *A guide to miRNAs in inflammation and innate immune responses.* FEBS J, 2018. **285**(20): p. 3695-3716.

242. Chakraborty, N., et al., *An integrated omics analysis: impact of microgravity on host response to lipopolysaccharide in vitro.* BMC Genomics, 2014. **15**: p. 659.

243. Schimmerling, W., *Genesis of the NASA Space Radiation Laboratory.* Life Sci Space Res (Amst), 2016. **9**: p. 2-11.

244. La Tessa, C., et al., *Overview of the NASA space radiation laboratory.* Life Sci Space Res (Amst), 2016. **11**: p. 18-23.

245. Miller, J. and C. Zeitlin, *Twenty years of space radiation physics at the BNL AGS and NASA Space Radiation Laboratory.* Life Sci Space Res (Amst), 2016. **9**: p. 12-18.

246. Cacao, E., et al., *Relative Biological Effectiveness of HZE Particles for Chromosomal Exchanges and Other Surrogate Cancer Risk Endpoints.* PLoS One, 2016. **11**(4): p. e0153998.

247. Cucinotta, F.A., *A new approach to reduce uncertainties in space radiation cancer risk predictions.* PLoS One, 2015. **10**(3): p. e0120717.

248. Shay, J.W., et al., *From mice and men to earth and space: joint NASA-NCI workshop on lung cancer risk resulting from space and terrestrial radiation.* Cancer Res, 2011. **71**(22): p. 6926-9.

249. Cucinotta, F.A., et al., *Space radiation cancer risks and uncertainties for Mars missions.* Radiat Res, 2001. **156**(5 Pt 2): p. 682-8.

250. Sridharan, D.M., et al., *Evaluating biomarkers to model cancer risk post cosmic ray exposure.* Life Sci Space Res (Amst), 2016. **9**: p. 19-47.

251. Beheshti, A., et al., *Age and space irradiation modulate tumor progression: implications for carcinogenesis risk.* Radiat Res, 2013. **179**(2): p. 208-20.

252. Beheshti, A., et al., *Proton irradiation augments the suppression of tumor progression observed with advanced age.* Radiat Res, 2014. **181**(3): p. 272-83.

253. Cui, M., et al., *Circulating MicroRNAs in Cancer: Potential and Challenge.* Front Genet, 2019. **10**: p. 626.

254. Wang, J., et al., *Lessons learned using different mouse models during space radiation-induced lung tumorigenesis experiments.* Life Sciences in Space Research, 2016. **9**: p. 48-55.

255. Vidyasekar, P., et al., *Genome Wide Expression Profiling of Cancer Cell Lines Cultured in Microgravity Reveals Significant Dysregulation of Cell Cycle and MicroRNA Gene Networks.* PLoS One, 2015. **10**(8): p. e0135958.

256. Grabham, P., et al., *Effects of ionizing radiation on three-dimensional human vessel models: differential effects according to radiation quality and cellular development.* Radiat Res, 2011. **175**(1): p. 21-8.

257. Gridley, D.S., et al., *Long-term changes in rat hematopoietic and other physiological systems after high-energy iron ion irradiation.* Int J Radiat Biol, 2008. **84**(7): p. 549-59.

258. Park, J.S., et al., *Ionizing radiation modulates vascular endothelial growth factor (VEGF) expression through multiple mitogen activated protein kinase dependent pathways.* Oncogene, 2001. **20**(25): p. 3266-80.

259. Fuentes, T.I., et al., *Simulated Microgravity Exerts an Age-Dependent Effect on the Differentiation of Cardiovascular Progenitors Isolated from the Human Heart.* PLoS One, 2015. **10**(7): p. e0132378.

260. Camberos, V., et al., *Effects of Spaceflight and Simulated Microgravity on YAP1 Expression in Cardiovascular Progenitors: Implications for Cell-Based Repair.* Int J Mol Sci, 2019. **20**(11).

261. Tian, Y., et al., *The Impact of Oxidative Stress on the Bone System in Response to the Space Special Environment.* Int J Mol Sci, 2017. **18**(10).

262. Grimm, D., et al., *The impact of microgravity on bone in humans.* Bone, 2016. **87**: p. 44-56.

263. Kovanda, A., T. Rezen, and B. Rogelj, *MicroRNA in skeletal muscle development, growth, atrophy, and disease.* Wiley Interdiscip Rev RNA, 2014. **5**(4): p. 509-25.

264. Maffiuletti, N.A., et al., *Neuromuscular Electrical Stimulation as a Potential Countermeasure for Skeletal Muscle Atrophy and Weakness During Human Spaceflight.* Front Physiol, 2019. **10**: p. 1031.

265. Bettis, T., B.J. Kim, and M.W. Hamrick, *Impact of muscle atrophy on bone metabolism and bone strength: implications for muscle-bone crosstalk with aging and disuse.* Osteoporos Int, 2018. **29**(8): p. 1713-1720.

266. Laurent, M.R., et al., *Muscle-bone interactions: From experimental models to the clinic? A critical update.* Mol Cell Endocrinol, 2016. **432**: p. 14-36.

267. Foessl, I., P. Kotzbeck, and B. Obermayer-Pietsch, *miRNAs as novel biomarkers for bone related diseases.* Journal of Laboratory and Precision Medicine, 2019. **4**.

268. Cheng, V.K., et al., *MicroRNA and Human Bone Health.* JBMR Plus, 2019. **3**(1): p. 2-13.

269. Materozzi, M., et al., *The Potential Role of miRNAs as New Biomarkers for Osteoporosis.* Int J Endocrinol, 2018. **2018**: p. 2342860.

270. Cappellesso, R., et al., *Spaceflight osteoporosis: current state and future perspective.* Endocr Regul, 2015. **49**(4): p. 231-9.

271. Jung, H.J., et al., *Comprehensive miRNA Profiling of Skeletal Muscle and Serum in Induced and Normal Mouse Muscle Atrophy During Aging.* J Gerontol A Biol Sci Med Sci, 2017. **72**(11): p. 1483-1491.

272. Suzuki, T. and J. Springer, *MicroRNAs in muscle wasting.* J Cachexia Sarcopenia Muscle, 2018. **9**(7): p. 1209-1212.

273. Zhang, S. and N. Chen, *Regulatory Role of MicroRNAs in Muscle Atrophy during Exercise Intervention.* Int J Mol Sci, 2018. **19**(2).

274. Chen, Z., M.G. Bemben, and D.A. Bemben, *Bone and muscle specific circulating microRNAs in postmenopausal women based on osteoporosis and sarcopenia status.* Bone, 2019. **120**: p. 271-278.

275. Fulzele, S., et al., *Muscle-derived miR-34a increases with age in circulating extracellular vesicles and induces senescence of bone marrow stem cells.* Aging (Albany NY), 2019. **11**(6): p. 1791-1803.

276. Hargens, A.R. and L. Vico, *Long-duration bed rest as an analog to microgravity.* J Appl Physiol (1985), 2016. **120**(8): p. 891-903.

277. Grassi, B., *Bed Rest Studies as Analogs of Conditions Encountered in Space and in Diseases.* Med Sci Sports Exerc, 2018. **50**(9): p. 1907-1908.

278. Sundblad, P., et al., *Standardization of bed rest studies in the spaceflight context.* J Appl Physiol (1985), 2016. **121**(1): p. 348-9.

279. Li, K., et al., *Screening and identification of novel mechanoresponsive microRNAs in rat femur under simulated microgravity.* Acta Astronautica, 2018. **153**: p. 166-173.
280. Wang, Y., et al., *MicroRNA-139-3p regulates osteoblast differentiation and apoptosis by targeting ELK1 and interacting with long noncoding RNA ODSM.* Cell Death Dis, 2018. **9**(11): p. 1107.
281. Arfat, Y., et al., *miR-208a-3p Suppresses Osteoblast Differentiation and Inhibits Bone Formation by Targeting ACVR1.* Mol Ther Nucleic Acids, 2018. **11**: p. 323-336.
282. Guller, I. and A.P. Russell, *MicroRNAs in skeletal muscle: their role and regulation in development, disease and function.* J Physiol, 2010. **588**(Pt 21): p. 4075-87.
283. Wang, F., et al., *Serum miRNAs miR-23a, 206, and 499 as Potential Biomarkers for Skeletal Muscle Atrophy.* BioMed Research International, 2017. **2017**: p. 9.
284. De Gasperi, R., et al., *The Signature of MicroRNA Dysregulation in Muscle Paralyzed by Spinal Cord Injury Includes Downregulation of MicroRNAs that Target Myostatin Signaling.* PLoS One, 2016. **11**(12): p. e0166189.
285. Rezen, T., et al., *Expression changes in human skeletal muscle miRNAs following 10 days of bed rest in young healthy males.* Acta Physiol (Oxf), 2014. **210**(3): p. 655-66.
286. Cucinotta, F.A., et al., *Space radiation risks to the central nervous system.* Life Sciences in Space Research, 2014. **2**: p. 54-69.
287. Kokhan, V.S., et al., *Risk of defeats in the central nervous system during deep space missions.* Neuroscience & Biobehavioral Reviews, 2016. **71**: p. 621-632.
288. Cekanaviciute, E., S. Rosi, and S.V. Costes, *Central Nervous System Responses to Simulated Galactic Cosmic Rays.* Int J Mol Sci, 2018. **19**(11).
289. Van Ombergen, A., et al., *The effect of spaceflight and microgravity on the human brain.* J Neurol, 2017. **264**(Suppl 1): p. 18-22.
290. Jandial, R., et al., *Space-brain: The negative effects of space exposure on the central nervous system.* Surg Neurol Int, 2018. **9**: p. 9.
291. Parihar, V.K., et al., *What happens to your brain on the way to Mars.* Sci Adv, 2015. **1**(4).
292. Cao, D.D., L. Li, and W.Y. Chan, *MicroRNAs: Key Regulators in the Central Nervous System and Their Implication in Neurological Diseases.* Int J Mol Sci, 2016. **17**(6).
293. Cho, K.H.T., et al., *Emerging Roles of miRNAs in Brain Development and Perinatal Brain Injury.* Front Physiol, 2019. **10**: p. 227.
294. Jin, X.F., et al., *Circulating microRNAs: a novel class of potential biomarkers for diagnosing and prognosing central nervous system diseases.* Cell Mol Neurobiol, 2013. **33**(5): p. 601-13.
295. Rao, P., E. Benito, and A. Fischer, *MicroRNAs as biomarkers for CNS disease.* Front Mol Neurosci, 2013. **6**: p. 39.
296. Sun, P., et al., *MicroRNA-based therapeutics in central nervous system injuries.* J Cereb Blood Flow Metab, 2018. **38**(7): p. 1125-1148.
297. Gaudet, A.D., et al., *MicroRNAs: Roles in Regulating Neuroinflammation.* Neuroscientist, 2018. **24**(3): p. 221-245.

298. Bai, M., et al., *Abnormal hippocampal BDNF and miR-16 expression is associated with depression-like behaviors induced by stress during early life.* PLoS One, 2012. **7**(10): p. e46921.

299. Zhao, Y., A.I. Pogue, and W.J. Lukiw, *MicroRNA (miRNA) Signaling in the Human CNS in Sporadic Alzheimer's Disease (AD)-Novel and Unique Pathological Features.* Int J Mol Sci, 2015. **16**(12): p. 30105-16.

300. Jayadev, S., et al., *Presenilin 2 influences miR146 level and activity in microglia.* J Neurochem, 2013. **127**(5): p. 592-9.

301. Dickson, J.R., et al., *Alternative polyadenylation and miR-34 family members regulate tau expression.* J Neurochem, 2013. **127**(6): p. 739-49.

302. Dorval, V., P.T. Nelson, and S.S. Hebert, *Circulating microRNAs in Alzheimer's disease: the search for novel biomarkers.* Front Mol Neurosci, 2013. **6**: p. 24.

303. Zendjabil, M., *Circulating microRNAs as novel biomarkers of Alzheimer's disease.* Clinica Chimica Acta, 2018. **484**: p. 99-104.

304. Shi, Y., et al., *MiR-21 is continually elevated long-term in the brain after exposure to ionizing radiation.* Radiat Res, 2012. **177**(1): p. 124-8.

305. Lee, A.G., et al., *Space flight-associated neuro-ocular syndrome (SANS).* Eye (Lond), 2018. **32**(7): p. 1164-1167.

306. Lee, A.G., et al., *Space Flight-Associated Neuro-ocular Syndrome.* JAMA Ophthalmol, 2017. **135**(9): p. 992-994.

307. Mader, T.H., et al., *Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight.* Ophthalmology, 2011. **118**(10): p. 2058-69.

308. Cucinotta, F.A., *Review of NASA approach to space radiation risk assessments for Mars exploration.* Health Phys, 2015. **108**(2): p. 131-42.