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Identification of gene and miRNA alterations in irradiated and non-irradiated human peripheral lymphocytes incubated in modeled microgravity

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Abstract. The combined presence of microgravity and ionizing radiation (IR) renders space environment dangerous for human health. To elucidate how human genome is affected by the combined action of these two physical agents, we analysed miRNA and mRNA expression profiles in human peripheral blood lymphocytes incubated in Modeled Microgravity (MMG) and in static condition 1g during the DNA damage response to IR. To improve the detection of functional miRNA-mRNA pairs, we performed gene expression profiles on the same samples assayed for miRNA profiling and we integrated miRNA and mRNA expression data. Our results evidenced alterations in miRNAs and genes belonging to the immune/inflammatory response and DNA-Damage response pathways. By integrating the transcriptome and microRNome, we evidenced that MMG affects the expression level of genes and miRNAs involved in the DNA damage response to IR. Notably, functional validation assays using luciferase reporter constructs confirmed miRNA-mRNA interactions derived from target prediction analyses.

Key words. Microgravity–Ionizing Radiation–Gene Expression–MiRNA expression profiling–DNA-Damage Response

1. Introduction

DNA damage response (DDR) is a complex pathway essential for cell survival in response to insults that danger DNA (Harper J.W. & Elledge S.J. 2007). Ionizing radiation (IR) causes direct DNA damage through single- and double-strand breaks (DSBs) and, in addition, indirect effects through radiolysis of water and reactive oxygen species formation (Azzam et al., 2012). DDR pathway has evolved to counteract DNA damage in ground gravity condition on Earth (1g), but in space environment, characterised by microgravity (10^{-2}g), cells experience a different status, which induces many adverse physiological changes in different tissues (Fitts et al., 2001; Narici et al., 2003; Carlsson et al., 2003; Sibonga et al., 2007; Ullrich et al., 2008; Crucian et al., 2008; Trappe et al., 2009), and render the cells more sensitive to IR-induced DNA damage. At the base of this evidence there are cell signalling alterations that in turn reflect on survival, chromosome aberrations, mutant frequency, cell cycle progression and apoptosis. Alterations at

genomic level have been reported in human peripheral blood lymphocytes (PBLs) exposed to spaceflight, but the limitations of experiments in real microgravity have pushed research studies towards conditions of simulated microgravity by using ground-based machines (such as clinostats and rotating wall vessel bioreactors) that generate a residual 10^{-3}-10^{-6}g force that approximates microgravity. In this context, our studies demonstrated that cell response to IR depends on environmental conditions occurring during DDR. In particular, when gamma-irradiated PBLs were incubated for 24 h in MMG, simulated by a ground-based rotating wall vessel (RWV) bioreactor, cell survival, DNA repair kinetics and apoptosis were significantly affected compared with PBLs incubated in 1g condition (Mognato and Celotti, 2005; Mognato et al. 2009). Functional validation studies demonstrated the miRNA-mRNA interactions selected, whereas experimental assays of cell viability and apoptosis induction validated the results obtained by bioinformatics analyses.

2. Results

2.1. MiRNA and mRNA expression changes in human PBLs incubated in MMG

We carried out microarray experiments of miRNA and mRNA profiling in human PBLs, isolated from healthy donors, incubated 4 and 24 h in 1g and in MMG after in vitro irradiation with 0.2 and 2 Gy of gamma-rays (Fig. 1). Non-irradiated PBLs were incubated in 1g and MMG, accordingly. Expression data showed that MMG alters miRNA and gene expression profiles of non-irradiated PBLs with respectively 42 differentially expressed miRNAs and 1581 genes (Girardi et al., 2014). The functional classification of miRNA correlated genes evidenced significant enrichment in the biological processes of immune/inflammatory response, signal transduction, regulation of response to stress, regulation of programmed cell death, and regulation of cell proliferation (not shown).

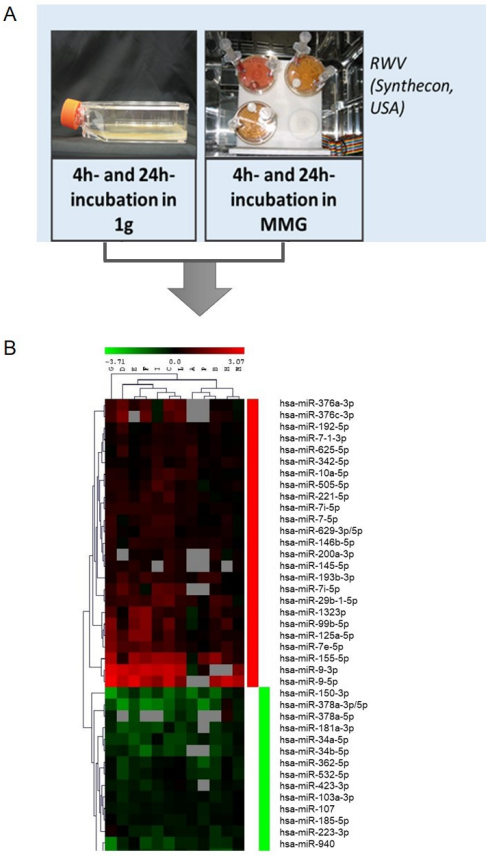

Fig. 1. Microarray experiments to assess miRNA and gene expression changes in human PBLs incubated in Modeled Microgravity (MMG). A) Microgravity was simulated by the Rotating Wall Vessel (RWV, Synthecon, Cellon, USA), placed inside a cell incubator. Ground based (1g) PBL cultures, both irradiated and non-irradiated, were kept at the same cell density in 75cm^2 flasks inside the incubator. At 4 and 24 h after irradiation total RNA was isolated from all PBL cultures and analyzed by using the "Human miRNA Microarray kit (V2) and the "Whole Human Genome Oligo Microarray (Agilent Technologies), respectively for miRNA and gene expression analyses. B) Representative dendrogram of differentially expressed miRNAs in non-irradiated PBLs incubated 24h in MMG.

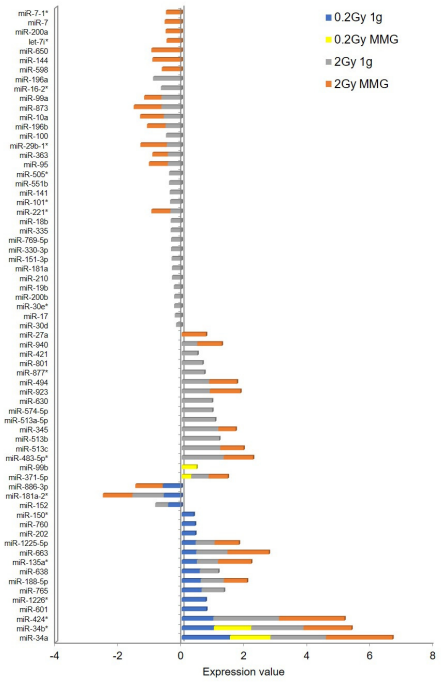

Fig. 2. Number of differentially expressed miRNAs in PBLs irradiated with 0.2 and 2 Gy and incubated for 4 and 24 h in MMG and in 1g condition.

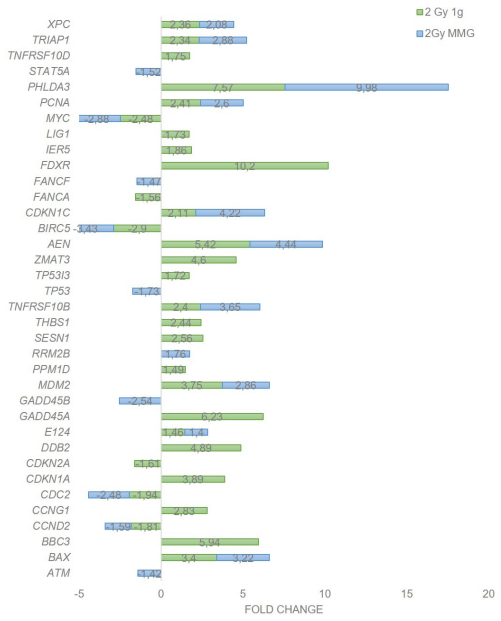

Fig. 3. Significantly anti-correlated genes of DDR pathway in 2Gy PBLs incubated 24 h in 1g or in MMG. A) Fold change is the mean of the expression values obtained from the transformed log2 (irradiated/non-irradiated) PBLs from microarray platform.

2.2. Identification of radioresponsive miRNAs targeting genes of DDR pathway

By microarray experiments we demonstrated that MMG alters miRNA expression profile of irradiated PBLs in a dose and time manner, but with a decreased radio responsiveness compared with in 1 g condition. At 4 h after irradiation with 0.2Gy we identified 16 miRNAs responsive in MMG-incubated PBLs (vs. 26 in 1 g) and 22 miRNAs responsive to 2Gy (vs. 20 in 1 g). After 24 h of incubation in MMG we identified 4 miRNAs responsive to 0.2Gy (vs. 17 in 1 g) and 32 miRNAs responsive to 2Gy (vs. 52 in 1 g) (Fig.2).

The 52 (in 1 g) and 32 (in MMG) differentially expressed miRNAs in 2Gy PBL resulted anticorrelated to a total of 379 and 391 transcripts, respectively (Girardi et al., 2021). To identify the most likely targets of differentially expressed miRNAs, we integrated miRNA and mRNA expression data obtained from the same donor. We focused on DDR pathway, evidencing differentially expressed genes anti-correlated to differentially expressed miRNAs of the same donors. In Fig.3 are shown genes of DDR pathway that are significantly anti-correlated with radio-responsive miRNAs. Several p53-related transcripts showed alterations in 2Gy-irradiated PBLs incubated for 24 h in MMG.

Microarray data from selected miRNAs and genes have been validated by qRT-PCR (not shown), then to ascertain that predicted miRNA-mRNA anti-correlations were real, we performed functional validation assays using luciferase reporter constructs that confirmed the selected miRNA-mRNA interactions (Fig.4).

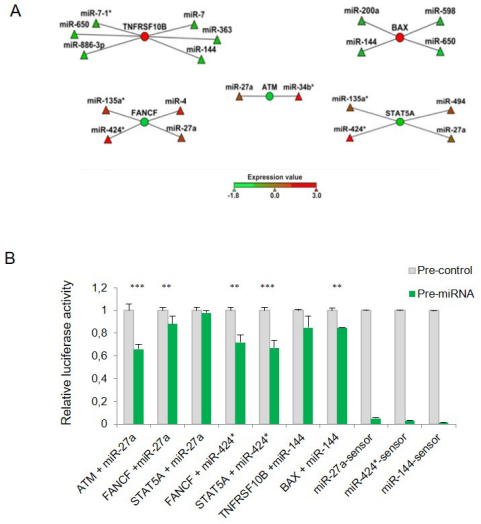

Fig. 4. Validation of miRNA-mRNA anti-correlations of DDR pathway. A) Cytoscape visualization of representative miRNA-mRNA anti-correlations. Circles represent transcripts and triangles miRNAs; the color scale attributes miRNA/mRNA expression level (green=down-regulation; red=up-regulation). (B) Functional validation analyses by luciferase assay on selected interactions. Results are mean ± SD of Firefly luciferase activity relative to controls, normalized on Renilla luciferase activity.

3. Conclusions

By microarray experiments we demonstrated that MMG alters miRNA expression profile of irradiated PBLs in a dose and time manner, but with a decreased radio responsiveness compared with in 1 g condition. Integration analyses of gene and miRNA expression profiling evidenced alterations in DDR pathway, indicating that modeled microgravity affects the cellular response of human PBLs to radio-induced DNA damage. Moreover, in non-irradiated PBLs, MMG alone affects significantly the expression level of miRNAs and genes involved in immune/inflammatory function regulation.

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