EVENT ABSTRACT

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# A comprehensive molecular and morphological study of the effects of space flight on human capillary endothelial cells: sample quality assessment and preliminary results.

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Human subjects and experimental animal models returning from Space show health issues related to the endothelium, a diffuse organ fundamental in maintaining body homeostasis. There are striking similarities between the effects of Space Flight (SF) on astronauts, and the consequences of old age, degenerative diseases and leading a sedentary life on Earth. Therefore, understanding the pathophysiological processes caused by SF might significantly advance prevention and treatment of frequent pathologies associated with ageing and lack of exercise.

Up to now, several cell types were characterized, although partially, for their response to SF, except endothelial cells (ECs) of capillary origin, a fundamental part of the endothelium. Therefore, we launched a human EC line from dermal capillary (HMEC-1, human Microvascular Endothelial Cells 1, CDC, USA) to the International Space Station (ISS): 'Endothelial cells' project, http://blogs.esa.int /iriss/2015/08/25/how-do-blood-vessel-cells-react-to-weightlessness/ (Soyuz 44S-42S launch campaign, September 2015) and undertook a cell and molecular biology study that included analysis of protein markers, of the transcriptome (RNASeq) and the methylome. Cells were seeded onto Cyclic Olefin Copolymer coverslips (IBIDI, Germany) coated with 2% pork skin gelatine, in MCDB131 medium supplemented with 20 mM HEPES (all reagents: Sigma-Aldrich, USA). Cell cultures were placed into micro-incubators (Experimental Units, EUs, Kayser Italia, Livorno, Italy) that, when connected to power, are able to fulfill under electronic control and without human intervention a scientific protocol of cell culture and fixation 2. We prepared 40 EUs: 24, were integrated on the Soyuz rocket and reached the ISS, 16 others remained on Earth to serve as ground controls (GC), (Figure 1, Table 1). The study required six-days of culturing in space, within KUBIK onboard ISS, with two cell growth medium changes, and a final fixation step. The short-duration mission foresaw a 6-hours rendez-vous flight to the ISS that instead, for unpredicted reasons, lasted 48 hours. Only afterwards, the samples were integrated into KUBIK. In parallel, we activated the GC EUs within the KISS facility in Pisa. Due to the delay, the medium change within KUBIK and KISS was still activated twice but after two instead of three days each time. At the end of space mission, the science material was returned via a commercial flight to Pisa, traveling in the ESA Thermocase B5 (Yellow Box). Cell culturing procedures, fixation methods and feasibility of planned experiments had been extensively tested during the experiment definition and experiment sequence test phases of the project 2,3. However, due to the operational complexity of the whole study, when the samples arrived at Pisa, before undertaking the molecular analyses, we performed a quality assessment of fixed samples whose results we present here with some preliminary data.

Initially, we evaluated the cell general morphology with light-microscopy observation. All coverslips (except GM03, GM08, FM05, FM18, that were somewhat less satisfactory) were found covered with cells (about 100% confluent) adherent and elongated (examples in Figure 2A). DNA (Figure 2B) and RNA were extracted and quantified. Each sample type, i.e. microgravity exposed (µ–g), centrifuge exposed (space-1g), and GCs, yielded in average about 500 ng total RNA, and about 1 µg genomic DNA.

Eight samples (Table 1) were used for whole-transcriptome RNASeq analysis (Applied Genomics Institute, IGA, Udine, Italy). Libraries were prepared with TruSeq Stranded Total RNA with Ribo-Zero Library Prep Kit (Illumina, San Diego, CA) and sequenced with Illumina HiSeq 2500 platform; CASAVA v1.8.2 was used for base calling. Quality of 2X125bp reads was assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw reads were aligned using STAR v2.5.3 4 to build version hg38 of the GENCODE human genome release 25. All samples had at least 44 millions PE reads uniquely aligned to the genome (Figure 2C). Counts for GENCODE basic annotated genes were calculated from the aligned reads using featureCounts function of the Rsubread R package 5. Normalization was carried out using edgeR R package 6. Raw counts were normalized according to library size to obtain counts per millions (CPM). Only genes with CPM greater than 1 in at least two samples were retained for subsequent analysis. Differential analysis was performed with voomWithQualityWeights function of limma R package 7, to down-weight outlier samples. Gene Set Enrichment Analysis (GSEA) 8 is in progress to determine whether the set of genes transcriptionally affected by microgravity or cosmic radiation was enriched in molecular pathways.

Eight samples (Table 1) were reserved for methylome analysis, currently in progress.

To evaluate cell morphology we began with F-actin staining because several reports describe the effect of microgravity 9–14, either real or simulated, on this protein in several cell types, so as to compare our own original results regarding microvascular ECs in real SF.

GCs showed well-defined F-actin stress and peripheral fibers whereas µ–g and space-1g cells showed collapsed F-actin networks (Figure 2D), with fluorescence intensity peaks considerably decreased (Figure 2D'). Also, stress granules (cytoplasmic structures that appear upon different stress stimuli 15,16) of different size were found in all space samples (Figure 2D, yellow arrow). These

similarities in both space sample types were possibly caused by the long rendez-vous flight, and/or other space-related factors, e.g. cosmic radiation, that were not counterbalanced by four days at 1g. The distribution of beta-catenin appeared less cytoplasmic and more in structured and oriented adherens junctions of μ-g cells compared to GCs (Figure 2E). HMEC-1 cells showed statistically significant change of morphology, measured with descriptors as circularity, roundness and solidity (Figure 2F) 17.
Altogether, these preliminary observations confirm that SF stresses ECs, damaging basic assets of cytoskeleton and transcriptome activity, whose dynamics are known to be strongly intertwined. The disorganization of F-actin seems counterbalanced by well-structured adherens junctions that preserve the endothelial sheet in SF.
For the first time, this work attempts at an integrated study of morphological, protein and transcriptome markers on a same biological system, specifically microvascular ECs to describe as integrally as possible their response to SF. On-going analysis will uncover further molecular details regarding the adaptive response of this fundamental component of physiological homeostasis.

Figure 1

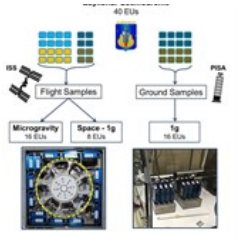



Figure 2

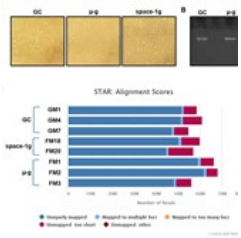
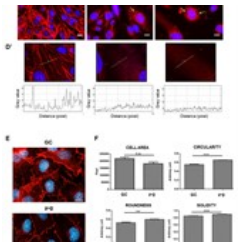



Figure 3



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