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ORIGINAL ARTICLE

Influence of Oxygen in the Cultivation of Human Mesenchymal Stem Cells in Simulated Microgravity: An Explorative Study

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Abstract Previous studies indicated that human Adipose Tissue-derived Mesenchymal Stem Cells (AT-MSCs) cultured in simulated microgravity (sim-μg) in standard laboratory incubators alter their proliferation and differentiation. Recent studies on the stem cell (SC) niches and the influence of oxygen on SC proliferation, senescence, and differentiation point to oxygen level as one of the key regulators of SC fate. Here we present the results of a study that focussed at the evaluation of the influence of oxygen level in the cultivation of AT-MSCs in sim-μg. In detail, cells were cultured for 14 days in sim-μg using the Random Positioning Machine (RPM) and two different oxygen concentrations: 5 % and 20 %. The results were compared with those obtained at 1g in the same conditions. Affymetrix Human Gene 1.0 ST array and Gene Ontology (GO) analysis were performed. The results confirmed that in all of the sim-μg experiments oxygen concentration modulates cell signalling and adhesion, in line with the knowledge that sim-μg affects cell shape and cytoskeletal organization.

Keywords Simulated microgravity · RPM · AT-MSCs · Niche · Oxygen · Gene ontology

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Abbreviations

AT-MSCs	Adipose Tissue-derived Mesenchymal Stem Cells
DAVID	Database for Annotation, Visualization, and Integrated Discovery
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
Sim-μg	Simulated microgravity
MSCs	Mesenchymal Stem Cells
RPM	Random Positioning Machine
SC	Stem Cells
g	unit gravity (9.81 m/s^2)

Introduction

Mesenchymal stem cells (MSCs) can be defined as cells having high proliferative potential with the capability of self-renew. They are undifferentiated, but can differentiate into the majority of marrow stromal cell lineages, including chondrocytes, osteoblasts, fibroblasts, adipocytes, endothelial cells, and myocytes. They reside within bone marrow, umbilical cord blood,

and other mesodermal tissues such as bone, adipose, muscle and tendon. Because of their plasticity, they are particularly sensitive to their immediate environments. *In vivo*, human MSCs, including those derived from adipose tissue (AT-MSCs), reside in specific "perivascular niches" (Moore and Lemischka 2006) in close association with cells, blood vessels and matrix glycoproteins. The three-dimensional space forming this architecture provides a highly specialized microenvironment in which contact and communication between these elements are critical for MSC self-renewal and multipotency. Even though MSCs are located close to vascular structures, the various tissues where these cells are found are characterized by a low oxygen tension (pO_2) of about 2–8 % (Kofoed et al. 1985; Harrison et al. 2002; Matsumoto et al. 2005; Pasarica et al. 2009; Mohyeldin et al. 2010). The low pO_2has been interpreted as a source of selective advantages, one of the most important being the possibility of escaping the DNA damage due to the generation of reactive oxygen species (ROS). Noteworthy it has been shown that pO_2 plays a key role in regulating SC fate (Csete 2005; Panyukhin et al. 2008). Nevertheless, SCs like most of the other cells are typically cultured in traditional incubators under 20 % O_2 (Csete 2005).

Considering the role of the "reduced pO_2" on the biology of human MSCs at 1 g, many results have been presented (Wang et al. 2005; D'Ippolito et al. 2006; Malladi et al. 2006, 2007; Zhu et al. 2006; Fehrer et al. 2007; Grayson et al. 2007; Xu et al. 2007; Rosova et al. 2008; Holzwarth et al. 2010; Lee et al. 2010). Some of these studies are occasionally controversial possibly due to variability in MSC donors, isolation, culture conditions, and experimental designs. On the contrary, no data have been published on the effects of "reduced pO_2" in the cultivation of human MSCs in simulated microgravity (sim-μg). In the present study, within the general aim of evaluating the effects of sim-μg on cultured human AT-MSCs, we intend to investigate if different O_2 tensions significantly modulate gene expression profiles.

Materials and Methods

AT-MSC Isolation, Characterization and Culture

AT-MSCs (one human donor, low passage number) were isolated and analyzed by fluorescence activated cell sorting (FACS) for the expression of the MSC surface markers CD166/ALCAM and CD105/endoglin as previously described (Zuk et al. 2002). Cells were cultured in DMEM supplemented with 500 μg/mL streptomycin sulphate, 600 μg/mL penicillin, 10 U/mL heparin, and 5 % platelet lysate. For pre-cultures AT-MSCs were seeded at 1.5–2 × 10^4 cells per 25-cm^2 in T25 culture flasks and cultured in a traditional incubator supplied by room air and buffered with 5 % CO_2. Then, cells were seeded in OptiCells™ fully filled with the medium previously degassed and then enriched with the selected oxygen concentration (5 % or 20 %). Cells were cultured for 14 days at 1 g or in sim-μg.

Experimental Protocol

To simulate microgravity, we used the Random Positioning Machine (RPM), a device in which the gravity vector is continually reoriented with direction and speed randomization (maximum velocity of 60°/s). In our conditions the maximum 'residual g' is expected to be 10^{-4} g (Klaus 2001; van Loon 2007; Borst and van Loon 2009). OptiCells™ were accommodated inside sealed metallic vessels filled either with a 5 % CO_2/air mixture (O_2 concentration in the range 20–21 %) or 5 % CO_2: 5 % O_2: 90 % N_2 mixture and subjected to sim-μg or accommodated on the bottom platform of the RPM (static controls, 1 g). At day 7 medium was quickly changed stopping the RPM for no longer than 5 min.

In detail, four experimental conditions were considered: 1) 1 g at 5 % O_2; 2) 1 g at 20 % O_2; 3) sim-μg at 5 % O_2; 4) sim-μg at 20 % O_2.

RNA Isolation, Target Preparation, and DNA Microarray Hybridization

At the end of the experiment cells were harvested and total RNA was immediately extracted using Trizol reagent (Invitrogen®) followed by a Qiagen RNeasy clean-up procedure, according to manufacturer's instructions. RNA purity was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

Biotin-labelled cDNA targets were synthesized starting from 150 ng of total RNA. Double stranded cDNA synthesis and related cRNA were performed with Ambion® WT Expression Kit (Ambion, Austin, TX). With the same kit the sense strand cDNA was synthesized before being fragmented and labelled with Affymetrix GeneChip® WT Teminal Labelling Kit (Affymetrix, Santa Clara, CA). Each eukaryotic GeneChip® probe array contains probe sets for several *B. subtilis* genes that are absent in the samples analyzed (*lys, phe, thr*, and *dap*). This Poly-A RNA Control Kit contains *in vitro* synthesized, polyadenylated

transcripts for these *B. subtilis* genes that are pre-mixed at staggered concentrations to allow GeneChip® probe array users to assess the overall success of the assay. Poly-A RNA controls final concentration in each target are *lys* 1:100,000; *phe* 1:50,000; *thr* 1:25,000; and *dap* 1:6,667.

Hybridization was performed using the GeneChip® Hybridization, Wash and Stain Kit. It contains mix for target dilution, DMSO at a final concentration of 7 % and pre-mixed biotin-labelled control oligo B2 and bioB, bioC, bioD and cre controls (Affymetrix cat #900299) at a final concentration of 50 pM, 1.5 pM, 5 pM, 25 pM and 100 pM, respectively. Targets were diluted in the hybridization buffer at a concentration of 25 ng/µl. A single GeneChip® Human Gene 1.0 ST was then hybridized with each biotin-labelled sense target. GeneChip® cartridges were washed and stained with GeneChip® Hybridization, Wash and Stain Kit in the Affymetrix Fluidics Station 450 following the FS450_0007 standard protocol.

Image Acquisition & Processing, and Bioinformatics

GeneChip arrays were scanned using an Affymetrix GeneChip® Scanner 3000 7G using default parameters. Affymetrix GeneChip® Command Console software was used to acquire GeneChip® images and generate .DAT and .CEL files.

Affymetrix.CEL files were imported in Partek Genomic Suite 6.5 software and pre-processed with Robust Multichip Analysis (Bolstad et al. 2003). An ANOVA statistical test was used to describe the experimental model, remove batch effects, and compute fold changes. In order to characterize the complete dataset and to investigate and categorize the gene ontology (GO)-annotations (molecular functions, biological processes, and pathways), DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/) was used (Dennis et al. 2003). P values ≤ 0.01 were considered statistically significant.

Results and Discussion

Human AT-MSCs were cultured for 14 days in sim-µg using two different oxygen concentrations: a) 20 %, the standard laboratory condition for cell cultures, and b) 5 %, that mimics the MSC niche pO_2. The results were compared with those obtained at 1 g in the same conditions. At the end of the experiments, global gene-expression profiling was performed using the Affymetrix Human Gene 1.0 ST Array. The following cross comparisons were made evaluating the effect of modulating: 1) pO_2 (5 % vs. 20 %) at different g levels and 2) gravity (sim-µg vs. 1 g) at different O_2 tensions. The four gene lists were cross compared and filtered on a 2-fold change cut-off. Then, data in terms of GO classification were evaluated using DAVID program.

The Effects of Reduced pO_2 at Different g Levels

The reduced pO_2 induces major gene modulations at both g levels. Out of 28, 869 genes, 462 were found significantly modulated at 1 g 5 % O_2 as compared to 1 g 20 % O_2. The same conditions resulted in 410 modulated genes in sim-µg. The two gene lists were intersected and an overlap of 125 genes, 90 % of them concordantly modulated, was found (Fig. 1).

The overlapping genes can be interpreted as markers of the effects of the reduced pO_2 *per se*, while the non overlapping genes can represent the gravity-dependent cell responses to the reduced pO_2. The Functional Annotation Clustering tool provided by DAVID grouped the common genes in 30 clusters, most of them predictable, among which those with higher score were: response to ROS/oxidative stress, cell morphology/motion, response to nutrient/organic substances, bone and skeletal development, and ion binding (for gene lists see Electronic Supplementary Material, Table 1).

Looking at the non overlapping genes, representing the gravity-dependent cell responses to the reduced pO_2, the GO analysis revealed a significant enrichment for genes with binding or peptidase activities. Whereas cell proliferation and cell adhesion were common features, although at different extent, at 1g most of the modulated genes were involved in the response to organic substances, cell to cell signalling, and oxidation-reduction processes, while in sim-µg were

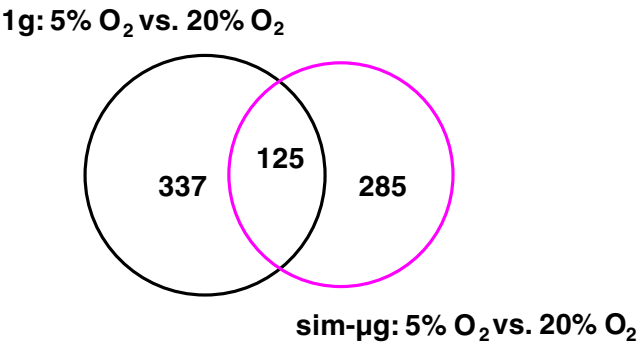

Fig. 1 VENN DIAGRAM of the number of significantly modulated genes at 5 % O_2 vs. 20 % O_2at 1 g (*black circle*) and in sim-µg (*purple circle*), and their overlap

related to wounding, cell motion and organization, and inflammation. In an attempt to shed light on the cellular responses *in toto* we evaluated the top pathways in which all of the modulated genes were involved. At 1 g the genes were mainly found to cluster in carbohydrate and lipid metabolism pathways, cancer (interpreted as "regulation of cell proliferation") and cardiovascular disease; while in sim-μg the genes were found to cluster in signalling molecules and interaction, cell communication, immune and endocrine systems (Fig. 2).

As expected, at 1 g, the reduced pO_2 affects essentially cell metabolism and proliferation, while in sim-μg it appears to modulate mainly cell communication and interaction processes, strictly connected with the cell cytoskeleton organization, which is very sensitive to environmental changes. Indeed, on the basis of what is known so far, microgravity implies critical cytoskeletal rearrangements that have always been observed in the different cell types cultured in simulated and real microgravity (Walther et al. 1996; Hughes-Fulford and Lewis 1996; Lewis et al. 1998; Ulbrich et al. 2011; Nabavi et al. 2011). Simulated microgravity-dependent cytoskeletal rearrangements have been also reported for human MSCs (Meyers et al. 2005).

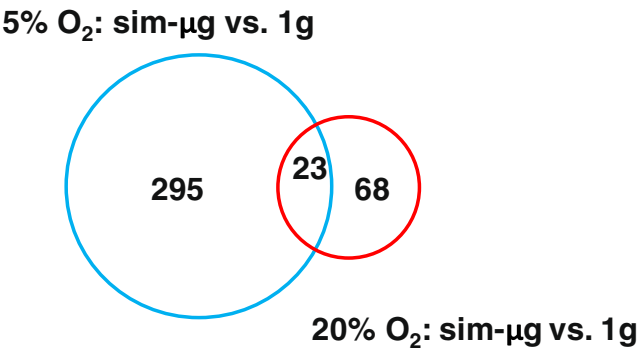

Fig. 3 VENN DIAGRAM of the number of significantly modulated genes in sim-μg vs. 1 g at 5 % O_2 (*blue circle*) and at 20 % O_2 (*red circle*), and their overlap

The Effects of Altered Gravity at Different O_2 Levels

Looking at the effects of sim-μg at both O_2 concentrations, in general less genes were found significantly modulated: out of 28, 869 genes, 318 at 5 % O_2 and 91 at 20 % O_2, with an overlap of only 23 genes, 80 % of them discordantly modulated (Fig. 3).

Since it is known that oxygen can influence human MSC phenotype, self-renewal and differentiation

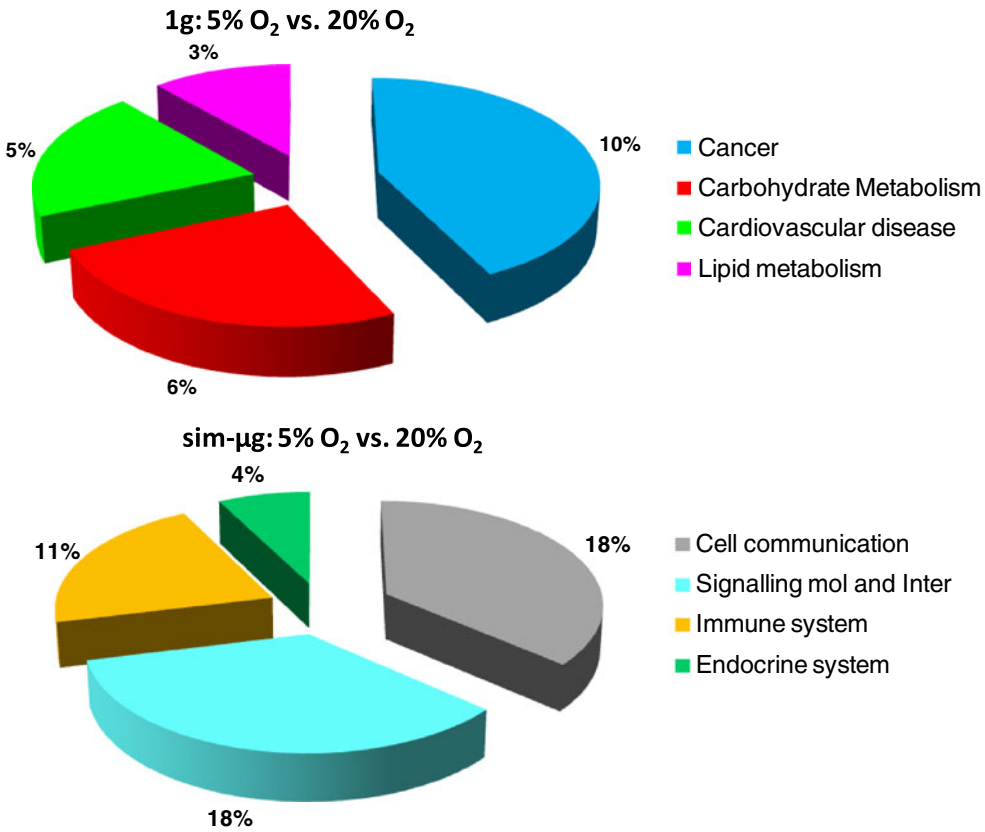

Fig. 2 High scored KEGG (http://www.genome.jp/kegg) pathways. *Upper panel*: 1 g 5 % O_2 vs. 20 % O_2; *lower panel*: sim-μg 5 % O_2 vs. 20 % O_2. The percentages are the total hits, divided by the number of annotated genes for the category

(Wang et al. 2005; D'Ippolito et al. 2006; Malladi et al. 2006, 2007; Zhu et al. 2006; Fehrer et al. 2007; Grayson et al. 2007; Xu et al. 2007; Rosova et al. 2008; Holzwarth et al. 2010; Lee et al. 2010), we first considered the expression and possible modulations of some human MSC specific markers. At both pO_2 cells expressed but did not modulate VCAM1, NT5E, ITGAL, CD34, encoding surface antigens commonly identified during human MSC isolation, and did not express CD45, CD31, and CD117 as expected. Most of the typical human MSC self-renewal and maintenance markers such as FGFs, OCT4, SOX2, REX1 and LIF, although expressed, were not significantly modulated in both conditions.

The overlapping genes could not be interpreted as markers of the effects of sim-μg (for gene list see Electronic Supplementary Material, Table 2). Indeed, although the 23 common genes were functionally annotated in clusters, the identified 3 clusters (wounding, defence, and inflammatory responses; programmed cell death; and ion binding), were not significant. Therefore, we decided to apply the GO analysis to the gene lists of the two conditions as the observed overlap was absent. The GO analysis revealed that the majority of modulated genes had binding activities at both O_2 concentrations (for details see Fig. 4). In addition, whereas cell adhesion and response to organic substances were common features, at 5 % O_2 most of the modulated genes were involved in oxidation-reduction processes, cell proliferation, and immune response (Fig. 5, upper panel), while at 20 % O_2 genes were present with higher percentage in the categories of response to wounding, signal transduction, inflammation, and cell communication and differentiation (Fig. 5, lower panel). It should be noted that the percentage of GO annotations can be above 100, due to the facts that genes may exhibit multiple functions, resulting in single gene being counted more than once (i.e., the genes are often present in more than one GO sub-database).

Major differences showed up when the modulated genes were clustered into pathways. Indeed, at 5 % O_2 the genes were mainly found to cluster in carbohydrate, lipid and cofactor/vitamin metabolism, and immune disease. At 20 % O_2 only 3 pathways can be identified and related to signalling molecules and interactions, endocrine and immune systems (Fig. 6).

In the light of the high donor to donor variability (*in vitro* growth rate, differentiation potential, responses to stimuli etc.), we are reluctant in discussing in details single gene regulation. In addition we are aware that the experiment has a number of limitations due to the restricted number of samples that can be al-

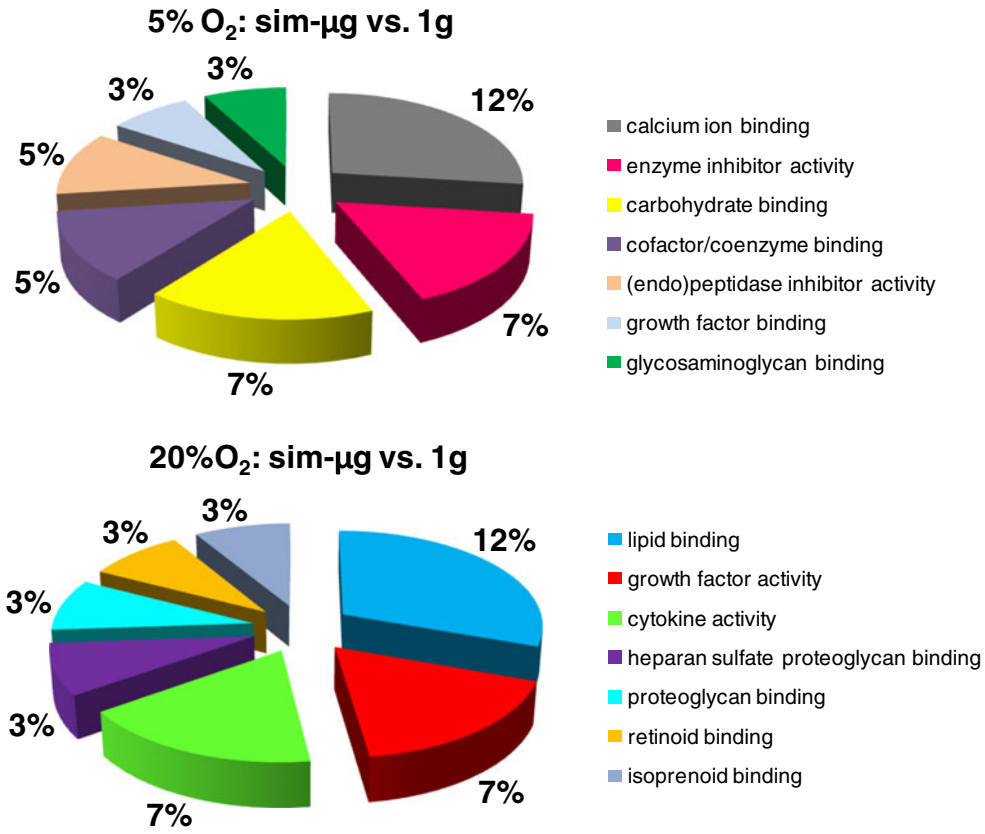

Fig. 4 GO analysis of the Molecular Functions. Top 7 categories with the most hits for Molecular Function are shown. *Upper panel*: sim-μg vs. 1 g at 5 % O_2; *lower panel*: sim-μg vs. 1 g at 20 % O_2. The percentages are the total hits, divided by the number of annotated genes for the category

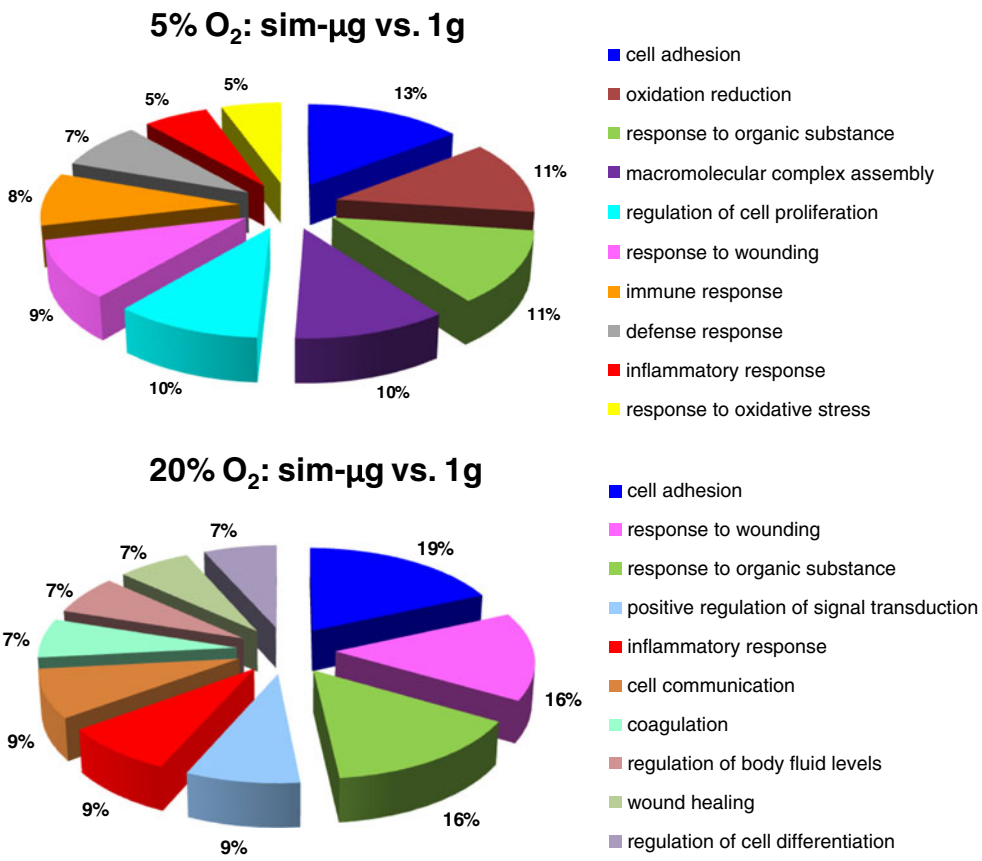

Fig. 5 GO analysis of Biological Processes. Top 10 categories with the most hits for Biological Processes are shown. *Upper panel*: sim-µg vs. 1 g at 5 % O_2; *lower panel*: sim-µg vs. 1 g at 20 % O_2. The percentages are the total hits, divided by the number of annotated genes for the category

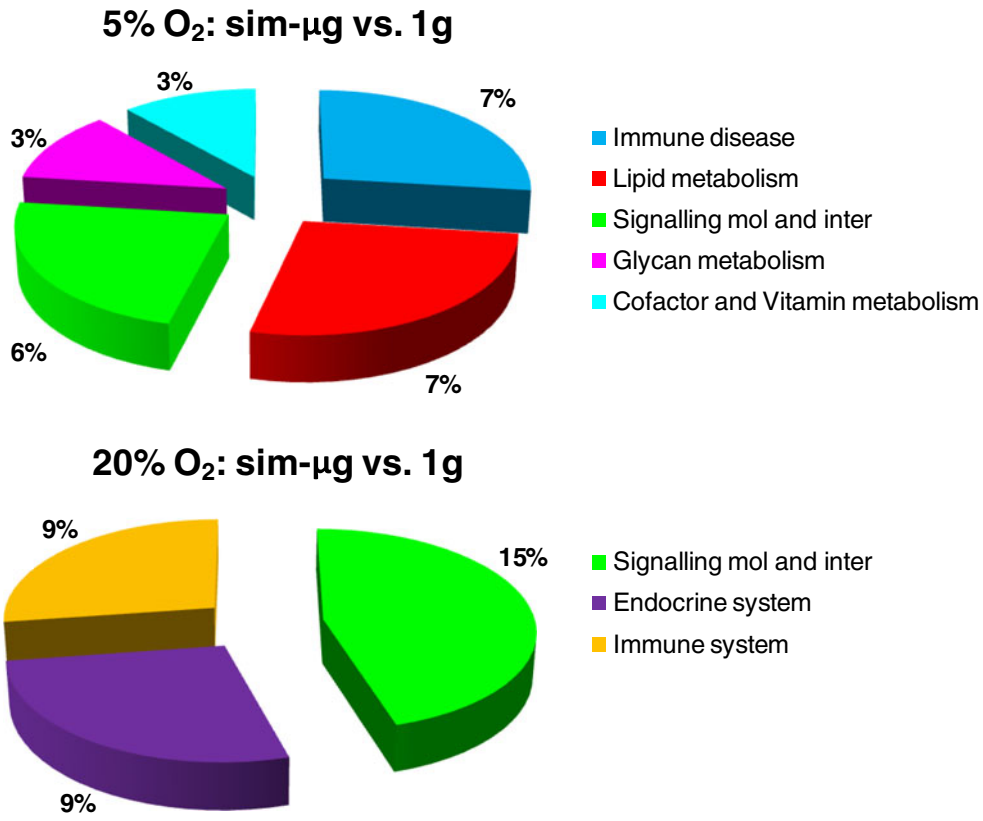

Fig. 6 KEGG pathways. *Upper panel*: sim-µg vs. 1 g at 5 % O_2; *lower panel*: sim-µg vs. 1 g at 20 % O_2. The percentages are the total hits, divided by the number of annotated genes for the category

located simultaneously on the RPM in gas controlled conditions.

Conclusion

The present study confirms that gene expression in simulated microgravity using the Random Positioning Machine is differently modulated depending on oxygen concentration. Indeed, as shown in the VENN diagram in Fig. 3 the two gene lists had small overlap not allowing the identification of significant common pathways.

Therefore, we have to restrict our conclusions to the following: adhesion and communication alterations are common features in all of the simulated microgravity experiments, thus reinforcing the knowledge that simulated microgravity affects the cytoskeletal organization, while oxygen seems to play a marked role in the degree of these alterations. Our results suggest that oxygen concentration must be taken into consideration performing experiments in simulated microgravity using sensitive systems such as human mesenchymal stem cells.

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References

Bolstad, B.M., Irizarry, R.A., Åstrand, M., Speed, T.P.: A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics **19**(2), 185–193 (2003)

Borst, A.G., van Loon, J.J.W.A.: Technology and developments for the random positioning machine, RPM. Microgravity Sci. Technol. **21**(4), 287–292 (2009)

Csete, M.: Oxygen in the cultivation of stem cells. Ann. NY Acad. Sci. **1049**, 1–8 (2005)

D'Ippolito, G., Diabira, S., Howard, G.A., Roos, B.A., Schiller, P.C.: Low oxygen tension inhibits osteogenic differentiation and enhances stemness of human MIAMI cells. Bone **39**(3), 513–522 (2006)

Dennis, G., Jr., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., Lempicki, R.A.: DAVID: database for annotation, visualization, and integrated discovery. Genome Biol. **4**(5), P3 (2003)

Fehrer, C., Brunauer, R., Laschober, G., Unterluggauer, H., Reitinger, S., Kloss, F., Gully, C., Gassner, R., Lepperdinger, G.: Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. Aging Cell **6**(6), 745–757 (2007)

Grayson, W.L., Zhao, F., Bunnell, B., Ma, T.: Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells. Biochem. Biophys. Res. Commun. **358**(3), 948–953 (2007)

Harrison, J.S., Rameshwar, P., Chang, V., Bandari, P.: Oxygen saturation in the bone marrow of healthy volunteers. Blood **99**(1), 394 (2002)

Holzwarth, C., Vaegler, M., Gieseke, F., Pfister, S.M., Handgretinger, R., Kerst, G., Muller, I.: Low physiologic oxygen tensions reduce proliferation and differentiation of human multipotent mesenchymal stromal cells. BMC Cell Biol. **11**, 11 (2010)

Hughes-Fulford, M., Lewis, M.L.: Effects of microgravity on osteoblast growth activation. Exp. Cell Res. **224**(1), 103–109 (1996)

Klaus, D.M.: Clinostats and bioreactors. Gravit. Space Biol. Bull. **14**, 55–64 (2001)

Kofoed, H., Sjontoft, E., Siemssen, S.O., Olesen, H.P.: Bone marrow circulation after osteotomy. Blood flow, pO2, pCO2, and pressure studied in dogs. Acta Orthop. Scand. **56**(5), 400–403 (1985)

Lee, S.H., Lee, Y.J., Song, C.H., Ahn, Y.K., Han, H.J.: Role of FAK phosphorylation in hypoxia-induced hMSCS migration: involvement of VEGF as well as MAPKS and eNOS pathways. Am. J. Physiol., Cell Physiol. **298**(4), C847–856 (2010)

Lewis, M.L., Reynolds, J.L., Cubano, L.A., Hatton, J.P., Lawless, B.D., Piepmeier, E.H.: Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). FASEB J. **12**(11), 1007–1018 (1998)

Malladi, P., Xu, Y., Chiou, M., Giaccia, A.J., Longaker, M.T.: Effect of reduced oxygen tension on chondrogenesis and osteogenesis in adipose-derived mesenchymal cells. Am. J. Physiol., Cell Physiol. **290**(4), C1139–1146 (2006)

Malladi, P., Xu, Y., Chiou, M., Giaccia, A.J., Longaker, M.T.: Hypoxia inducible factor-1alpha deficiency affects chondrogenesis of adipose-derived adult stromal cells. Tissue Eng. **13**(6), 1159–1171 (2007)

Matsumoto, A., Matsumoto, S., Sowers, A.L., Koscielniak, J.W., Trigg, N.J., Kuppusamy, P., Mitchell, J.B., Subramanian, S., Krishna, M.C., Matsumoto, K.: Absolute oxygen tension (pO(2)) in murine fatty and muscle tissue as determined by EPR. Magn. Reson. Med. **54**(6), 1530–1535 (2005)

Meyers, V.E., Zayzafoon, M., Douglas, J.T., McDonald, J.M.: RhoA and cytoskeletal disruption mediate reduced osteoblastogenesis and enhanced adipogenesis of human mesenchymal stem cells in modeled microgravity. J. Bone Miner. Res. **20**(10), 1858–1866 (2005)

Mohyeldin, A., Garzon-Muvdi, T., Quinones-Hinojosa, A.: Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell **7**(2), 150–161 (2010)

Moore, K.A., Lemischka, I.R.: Stem cells and their niches. Science **311**(5769), 1880 (2006)

Nabavi, N., Khandani, A., Camirand, A., Harrison, R.E.: Effects of microgravity on osteoclast bone resorption and osteoblast cytoskeletal organization and adhesion. Bone **49**(5), 965–974 (2011)

Panyukhin, N.V., Vishnyakova, K.S., Yegorov, Y.E.: Effect of partial oxygen pressure on survival, proliferation, and differentiation of mouse bone marrow mesenchymal stem cells. Biochem. **2**(4), 326–332 (2008)

Pasarica, M., Sereda, O.R., Redman, L.M., Albarado, D.C., Hymel, D.T., Roan, L.E., Rood, J.C., Burk, D.H., Smith,

S.R.: Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. Diabetes **58**(3), 718–725 (2009)

Rosova, I., Dao, M., Capoccia, B., Link, D., Nolta, J.A.: Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells **26**(8), 2173–2182 (2008)

Ulbrich, C., Pietsch, J., Grosse, J., Wehland, M., Schulz, H., Saar, K., Hubner, N., Hauslage, J., Hemmersbach, R., Braun, M., van Loon, J., Vagt, N., Egli, M., Richter, P., Einspanier, R., Sharbati, S., Baltz, T., Infanger, M., Ma, X., Grimm, D.: Differential gene regulation under altered gravity conditions in follicular thyroid cancer cells: relationship between the extracellular matrix and the cytoskeleton. Cell Physiol. Biochem. **28**(2), 185–198 (2011)

van Loon, J.J.W.A.: Some history and use of the random positioning machine, RPM, in gravity related research. Adv. Space Res. **39**(7), 1161–1165 (2007)

Walther, I., Bechler, B., Muller, O., Hunzinger, E., Cogoli, A.: Cultivation of Saccharomyces cerevisiae in a bioreactor in microgravity. J. Biotechnol. **47**(2–3), 113–127 (1996)

Wang, D.W., Fermor, B., Gimble, J.M., Awad, H.A., Guilak, F.: Influence of oxygen on the proliferation and metabolism of adipose derived adult stem cells. J. Cell. Physiol. **204**(1), 184–191 (2005)

Xu, Y., Malladi, P., Chiou, M., Bekerman, E., Giaccia, A.J., Longaker, M.T.: In vitro expansion of adipose-derived adult stromal cells in hypoxia enhances early chondrogenesis. Tissue Eng. **13**(12), 2981–2993 (2007)

Zhu, W., Chen, J., Cong, X., Hu, S., Chen, X.: Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. Stem Cells **24**(2), 416–425 (2006)

Zuk, P.A.P.A., Zhu, M.M., Ashjian, P.P., De Ugarte, D.A.D.A., Huang, J.I.J.I., Mizuno, H.H., Alfonso, Z.C.Z.C., Fraser, J.K.J.K., Benhaim, P.P., Hedrick, M.H.M.H.: Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell **13**(12), 4279–4295 (2002)