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Increased stemness and migration of human mesenchymal stem cells in hypoxia is associated with altered integrin expression

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ABSTRACT

Human mesenchymal stem cells (hMSCs) are regularly cultured and characterised under normoxic (21% O_2) conditions, although the physiological oxygen tension in the stem cell niche is known to be as low as 1–2%. Oxygen itself is an important signalling molecule, but the distinct impact on various stem cell characteristics is still unclear. Therefore, the aim of this study was to evaluate the influence of oxygen concentration on the hMSC subpopulation composition, cell morphology and migration on different surfaces (polystyrene, collagen I, fibronectin, laminin) as well as on the expression of integrin receptors. Bone marrow-derived hMSCs were cultured either in normoxic (21% O_2) or hypoxic (2% O_2) conditions. The hMSC subpopulations were assessed by aspect ratio and cell area. Hypoxia promoted a more homogeneous cell population with a significantly higher fraction of rapidly self-renewing cells which are believed to be the true stem cells. Under hypoxic conditions hMSC volume and height were significantly decreased on all surfaces as measured by white light confocal microscopy. Furthermore, low oxygen tension led to a significant increase in cell velocity and Euclidian distance on all matrixes, which was evaluated by time-lapse microscopy. With regard to cell-matrix contacts, expression of several integrin subunits was evaluated by semi-quantitative RT-PCR. Increased expression of the subunits α_1, α_3, α_5, α_6, α_{11}, α_v, β_1 and β_3 was observed in hypoxic conditions, while α_2 was higher expressed in normoxic cultured hMSCs. Taken together, our results indicate that hypoxic conditions promote stemness and migration of hMSC along with altering their integrin expression.

1. Introduction

In regenerative medicine, human mesenchymal stem cells (hMSCs) are commonly harvested from the bone marrow, where they reside in a stem cell niche [1]. Chemical and physical signals can either keep the cells in quiescence or induce their activation with respect to cell migration and differentiation [2]. In this niche the oxygen tension is known to be as low as 1–2% [1]. Although it has been recognised that oxygen exerts an important impact on the cells [3,4], hMSCs are mostly cultured at 21% O_2. Therefore, many observations with regard to hMSC migration, proliferation, stemness, and senescence have been obtained under these non-physiological conditions. Recent publications began to reveal distinct effects of reduced oxygen concentration on hMSCs. Cell proliferation was shown to be enhanced [5,6] colony forming potential seems to be increased and stemness is believed to be maintained longer by hypoxic culture conditions [1,3,5]. Furthermore, synthesis of extracellular matrix (ECM) may be accelerated, growth factors are upregulated [5] and hMSC differentiation capacity and migration can be altered, while results are inconsistent [1,5]. Yet, the incomplete understanding of the various factors influencing the stem cell characteristics of hMSCs hinders exploiting their real therapeutic potential.

Therefore, the aim of this study was to evaluate the influence of oxygen concentration on the hMSC subpopulation composition, cell morphology and migration on different extracellular matrix proteins as well as on the expression of integrin receptors. HMSCs do not represent a homogenous cell type, but rather consist of several subpopulations featuring different cell characteristics. Prockop et al. identified the subpopulation of rapidly self-renewing cells, as being stem cells in the most native state with the highest differentiation capacity [7]. For tissue engineering and cell-based therapies it is desirable to enhance the homogeneity of the cell

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culture and the portion of these uncommitted cells. To date there are only hints that the stemness of hMSCs is increased by hypoxic cell culture, as increased expression of pluripotency marker genes (Oct-4, Rex-1) has been observed [3,5]. In order to assess the influence of oxygen concentration on the percentage of rapidly self-renewing cells, we analysed the subpopulations by morphometric parameters over the entire culturing period of 64 days for both, normoxic (21% O_2) and hypoxic (2% O_2) conditions [8]. Moreover, as different ECM proteins also alter the hMSC morphology [9], we additionally examined the effect of oxygen concentration and collagen I, fibronectin and laminin on the cell morphology. Furthermore, cell migration is of utmost importance for cell-based tissue regeneration, since stem cells must reach the site of repair [10]. In order to assess the influence of oxygen concentration on cell migration, the velocity and the Euclidian distance were assessed on polystyrene as well as on collagen I, fibronectin, laminin in both culture conditions. Finally, since cell-matrix contacts, which play a pivotal role in cell migration, are mainly mediated by integrin receptors, we additionally screened for the expression of the subunits of the most prominent binding integrins for these ECM proteins using RT-PCR.

2. Material and methods

2.1. Human mesenchymal stem cells (hMSC)

HMSCs were obtained from the femoral bone marrow of a 43 years old healthy, male donor according to the assignment of the LMU Ethical Commission (Project Number 311/04), written consent was acquired. Mononuclear cells were separated by a Ficoll (polysucrose) density gradient and the cell pellet was resuspended in minimum essential medium, Alpha GlutaMAX (Invitrogen, Germany) supplemented with 10% foetal bovine serum (FBS, Sigma–Alrich, USA) and 40 IU/ml penicillin/streptomycin (PAA Laboratories GmbH, Austria). Cell suspension was then equally divided into two portions. One half was further cultivated using a standard humidified incubator (21% O_2, Hera cell 240, Thermo Scientifc, Germany). The other half was cultured using a hypoxic humidified incubator (2% O_2, MCO-5M, Sanyo, Germany), both at 5% CO_2 and 37 °C.

2.2. Surface marker analysis

According to the consensus of the International Society for Cellular Therapy by Dominici et al. MSC have to be positive for CD105, CD73 and CD90 ($\geqslant$95%) and negative for CD45, CD19 and HLA-DR ($\leqslant$2%). Antibodies against CD73-PE, CD90-PE-Cy5 and CD105- FITC were obtained from BD Biosciences, Germany; HLA-DR-APC from Beckman Coulter, Germany; CD19-APC and CD45-APC from Abcam, UK. 10,000 cells (passage 6) per CD-marker were labelled with 3 μl of primary fluorescently labelled antibodies or isotype controls and incubated for 20 min and washed in PBS. Fluorescence was measured by a fluorescence-activated cell sorting (FACS) device (FACSCalibur, BD-Bioscience, Germany). Data was analyzed using FlowJo FACS analysis software 7.6 (Treestar Inc., USA). Two independent experiments were performed.

2.3. Osteogenic differentiation

Osteogenic differentiation was performed as described earlier [4]. Briefly, 3000 cells/cm^2 (passage 2) were plated in a 6-well plate (NUNC, Germany) and expanded to 90% confluence. Differentiation was started using induction media. After 21 days cells were fixed and incubated with Alizarin Red solution (Osteogenesis Assay Kit, Merck-Millipore, USA) for 20 min. Histological images were acquired with a 10× phase-contrast objective mounted on an Axiovert 40 CFL microscope equipped with a RGB colour camera (both Zeiss, Germany). Alizarin Red quantification was performed according to the company's protocol. In brief, stained cells were incubated with 10% acetic acid for 30 min; and detached with a cell scraper and heated for 10 min at 85 °C. Following, optical density was measured at 405 nm using an ELISA reader (Multiskan FC, Thermo Scientific).

2.4. Adipogenic differentiation

Adipogenic differentiation was performed as described earlier [11]. In brief, 5000 cells/cm^2 (passage 2) were seeded in a 6-well plate and expanded to 100% confluence. Differentiation was started using induction media for 5 days, followed by conservation phase of 2 days using high-glucose-media. The induction/conservation cycle was repeated for 3 weeks. Next, cells were fixed with 4% paraformaldehyde (PFA) and stained with 0.2% Oil Red O solution. Subsequently, cells were imaged by phase-contrast microscopy as described above. For quantification of lipid content, AdipoRed-Adipogenesis-Assay-Kit (Lonza, USA) was used according to the manufacturer's protocol. After incubation with AdipoRed solution, fluorescence was measured using a UV/VIS multiplate reader (Safire, Tecan, Switzerland). For osteogenic and adipogenic differentiation two independent experiments, in triplicates, were performed.

2.5. Cell area, aspect ratio and cell volume

Using the ImageJ software's free hand tool, 100 cells per passage and culture condition were used for measurements of cell area and aspect ratio. Cells were imaged by phase-contrast microscopy as described above. For volume measurements, 1000 cells/cm^2 were grown on glass slides coated with collagen I (Col1), fibronectin (FN) or laminin (LN) (10 μg/ml protein) or polystyrene for 48 h [8]. Then, cells were fixed with 4% PFA, incubated in an ascending ethanol row up to 100% and subsequently, dehydrogenised with electronic grade tetramethylsilane (Sigma–Aldrich, Germany) before coating with 10 nm gold in a sputter coater (Bio-Rad, SCD 040, Germany). 25 cells per protein and culture condition were imaged using a white light confocal microscope (PLμ 2300, Sensofar, Spain) equipped with 50×/0.8 objective and a black/white camera (XC-HR58, Nikon, Germany). Data was analyzed using the open-source program Gwyddion. The curves in Fig. 2A/B represent trendlines as an appreciation of the curve progression.

2.6. Time-lapse analysis

hMSC (1000 cells/cm^2) were plated on 6-well dishes after coating with Col1, FN, LN or without coating. Directly after plating, the cells were imaged in 15 min intervals for 72 h with a black/white camera (AxioCam-MRm, Zeiss, Germany) mounted on an automated AxioVert-S100 (Zeiss, Germany) inverted microscope. For imaging in normoxia cells were maintained in a biochamber (Incubator XL S, Pecon, Germany) attached to the microscope. For hypoxia, a self-designed magnetic-sealed polymethylmetaacrylate box was used. Oxygen and carbon dioxide were controlled with a ProOx110 and ProCO_2 gas feedback devices (both from BioSpherix, USA). Hundred cells on each surface were evaluated for their velocity in μm/s using the plugin MTrackJ for ImageJ. Furthermore, the Euclidean distance, reflecting the shortest distance from start to end point, was visualised in a polar plot for 10 cells per protein and culture condition.

2.7. Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

The expression of integrin α-subunits 1, 2, 3, 5, 6, 11 and v, as well as the β-subunits 1, 3 and 5 were screened as described by Po-

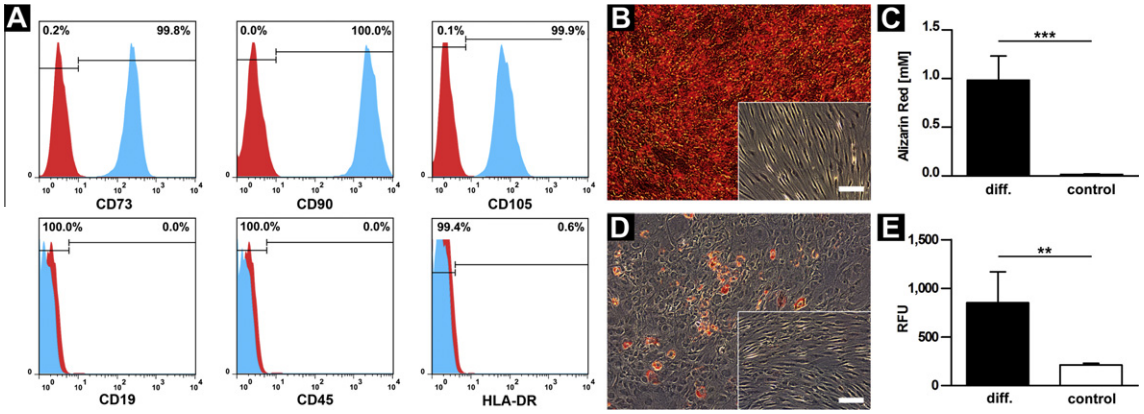

Fig. 1. Cells display stem cell characteristics. (A) Exemplary CD-expression (isotype controls: red, CD markers: blue). Bar = 200 μm, *** < 0.0001, ** < 0.001. (B) Representative Alizarin Red staining of osteogenically differentiated cells. (B-Insert) Controls were negative for Alizarin Red. (C) Quantification of Alizarin Red staining. (D) Representative Oil Red O staining following adipogenic differentiation. (D-Insert) Controls were negative for Oil Red O. (E) Quantification of lipid content, presented in relative fluorescence units (RFU). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

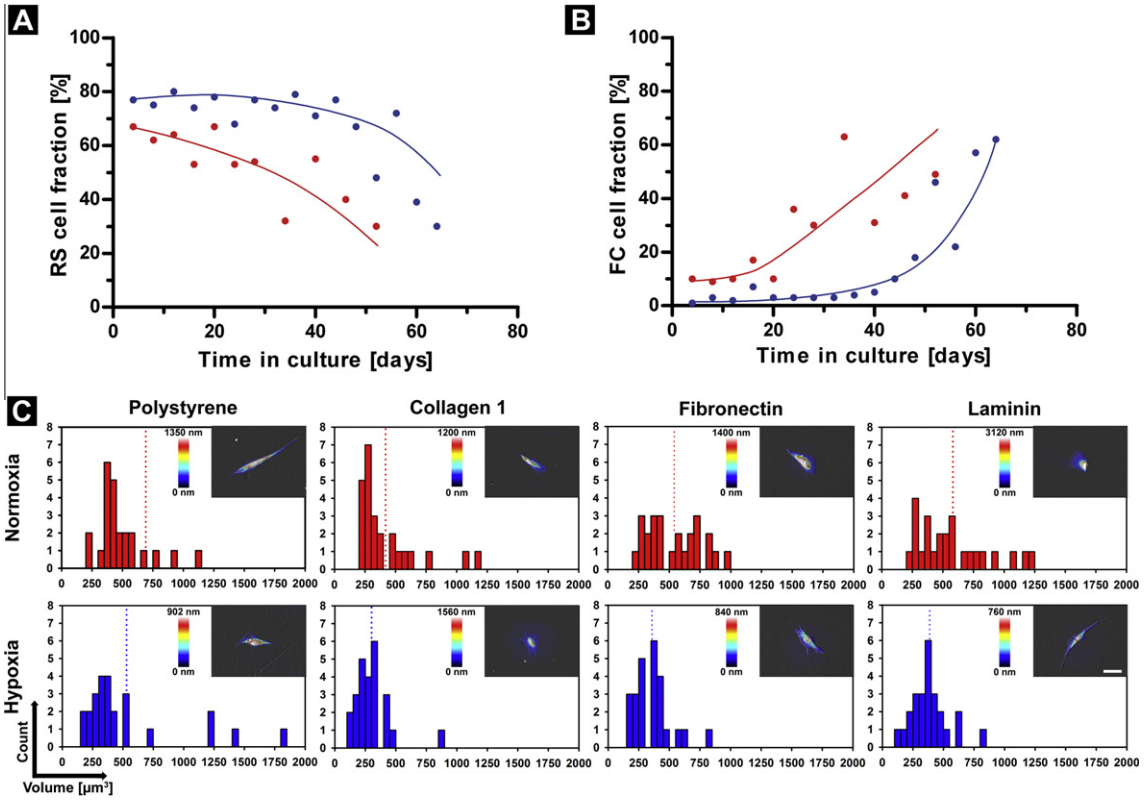

Fig. 2. Subpopulation analysis and volume measurements of normoxic (red) and hypoxic (blue) cultured cells (A) Fraction of RS cells (B) Fraction of FC cells. (A/B) Curves represent trendlines. (C) Histograms of cell volumes. Dashed line = median; inserts = exemplary white light confocal images, bar = 50 μm, colour bar = cell height. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pov et al. [12]. Briefly, RNA was isolated using the RNeasy Mini Kit (Qiagen, Germany) according to the manufactures protocol, and stored at −20 °C until cDNA synthesis. Prior PCR, cDNA synthesis was performed using a cloned AMV first strand cDNA synthesis kit (Invitrogen, Germany). The PCR reaction was performed with Taq DNA polymerase (Invitrogen, Germany) using a DNA Engine machine (Bio-Rad, Germany). Corresponding primers and PCR conditions are shown in Supplementary Table 1. Samples were normalised against the house keeping gene GAPDH. Relative expression levels were calculated by applying a densitometric quantification of band intensities as described earlier [4]. The integrin expression was normalised to GAPDH expression and results are presented as fold changes to normoxia. All integrin PCRs were repeated twice.

2.8. Statistical analysis

Quantitative data was analyzed using GraphPad Prism version 5.0 (Statcon, Germany). If samples showed normal distribution a Student's *t*-test, if not a Mann–Whitney U-test, was performed. A value of $p < 0.05$ was considered significant. Data is presented as mean ± standard deviation.

3. Results and discussion

3.1. Obtained cells feature mesenchymal stem cell characteristics

According to the minimal criteria for multipotent MSC, as defined by Dominici et al. [13], we could demonstrate that the isolated cells were plastic-adherent and expressed CD73, CD90, CD105 while lacked the expression of CD19, CD45 and HLA-DR (Fig. 1A). Furthermore, they were capable of differentiation into the osteogenic (Fig. 1B/C) and adipogenic (Fig. 1D/E) lineages. Hence, the stem cell character of the obtained cells could be validated.

3.2. Hypoxic cell culture conditions delay senescence, increase homogeneity of hMSCs and promote rapidly self-renewing cells

Normoxic cells went into senescence after 52 days, while cells in hypoxic culture went into senescence after 64 days (Supplementary Fig. 1). Cells were grouped into rapidly self-renewing (RS), spindle-shaped (SS), flattened (FC) and flattened spindle-shaped (FSS) cells, according to Haasters et al. [8]. Already after the first passage, hypoxic culture displayed a significantly higher proportion of RS cells (normoxia: 67%, hypoxia: 77%) and a significantly lower proportion of FC cells (normoxia: 10%, hypoxia: 1%) compared to normoxia. Over the course of normoxic cell culture the fraction of FC cells constantly increased starting from day 20 to reach 49% before going into senescence. Contrary, the fraction of RS cells continuously decreased to 30% at day 52 (Fig. 2A/B). In contrast, hypoxic hMSCs maintained their homogenous rapidly self-renewing morphology for up to 52 days, before decreasing to 30% at day 64. In analogy, FC cells remained constantly low for 52 days. Then, an increase was observed to 62% at day 64. Exemplary dot plots of the cell fractions are presented in Supplementary Fig. 2.

Haasters et al. correlated parameters of cell morphology to the different subpopulations, thereby the different cell types can be identified by measurement of area and aspect ratio [8]. The different subpopulations show diverging cell characteristics. FC and SS cells demonstrate reduced self-renewable capacity with the lowest belonging to FC cells [14]. In contrast, RS cells have the highest self-renewable capacity and the highest differentiation ability [7]. While RS cells express all three positive stem cell markers, FC lost part of the surface proteins and are believed to be osteogenic progenitors in early passages [8]. In later passages, however FC may represent entry into cell senescence [15]. This is in line with the findings of Grayson et al. who found differences in hMSC growth characteristics with varying oxygen levels. While normoxic cultured cells formed 'islets' with slowly growing densities, hypoxic hMSC showed increased proliferation forming multiple cell layers [3]. In regenerative medicine, a homogenous cell population with a high proliferation and differentiation capacity is needed. No study has evaluated the distribution of hMSC subpopulations yet. Furthermore, the time dependent development of the cell population has not been studied up to date. We could demonstrate that hypoxic culture conditions increase the fraction of RS cells and increase homogeneity of hMSC. Consequently, since RS cells are ideal for cell therapy [7], culture conditions such as hypoxia, promoting RS cells are desirable.

3.3. Cell height and volume are reduced in hypoxic environment

To precisely describe hMSC morphological changes, we further measured the cellular volume and height of 25 cells cultured on polystyrene as well as on Col1, FN and LN. Height on all surfaces was lower in hypoxia compared to normoxia, although only cells cultured on laminin coating revealed significant differences ($p = 0.0254$). Cell height within both culture conditions was the least on fibronectin, increasing to polystyrene and collagen to reach a maximum on laminin. Differences were only significant for normoxic cells on laminin when compared to polystyrene ($p = 0.022$). Hypoxic cells again showed a higher homogeneity on all surfaces. Histograms of cell volumes and exemplary topographical images are presented in Fig. 2C, while quantification in details in Supplementary Table 2.

Regarding cell volume, hypoxic conditions also led to a reduction on all surfaces compared to normoxia, with significant differences for fibronectin ($p = 0.0038$) and laminin ($p = 0.0123$). The maximum volume for both culture conditions was obtained when hMSC were cultured on polystyrene surface. In contrast, while coating with collagen lead to the most pronounced reduction of volume in both culture conditions when compared to polystyrene (normoxia: $p = 0.0153$, hypoxia: $p = 0.0096$). Histograms of the cell volumes are presented in Fig. 2C. The impact of the culture surface on the cell volume was more pronounced in hypoxia.

Cell height and volume are influenced by various factors, such as cell adhesion, cytoskeletal arrangement and locomotion [16]. Therefore, we examined the influence of several ECM proteins found in the bone marrow. Our results reveal a reduction of height and volume for hypoxic cells compared to the normoxic for all substrates. Thus, our results are the first hint, that oxygen level and culture surface affect cell height and volume of hMSC, presumably due to cytoskeletal rearrangement in the cells.

3.4. Hypoxia affects migration of hMSCs

We investigated the influence of the oxygen level on cell migration on polystyrene, and on different ECM proteins by time-lapse analysis. In sum 1019, cell tracks with 105000 data points were evaluated. The results from normoxic culture conditions were considered 100% and set as control. For all surfaces, a significant increase of mean migration velocity was observed in hypoxic conditions, when compared to normoxia ($p \leqslant 0.0063$) Fig. 3A. This effect was most pronounced on polystyrene (164.0% ± 43.2%), while collagen I and fibronectin, led to an intermediate increase of velocity of 1.4-fold. The slightest increase was observed on fibronectin (112.2% ± 35.8%). In normoxia, all ECM proteins led to a significant increase of cell velocity when compared to polystyrene. In hypoxia only laminin and collagen showed significant differences compared to polystyrene.

The Euclidian distance, reflecting the shortest distance from start to end point, was nearly doubled for polystyrene, collagen I and laminin coating in hypoxic conditions, while fibronectin coating did not show major differences when compared to normoxic culture conditions. Interestingly, in hypoxia, collagen I and laminin coating led to a remarkable increase of the Euclidian distance compared to polystyrene. In normoxia for all three ECM proteins the increase of the Euclidian distance was much less pronounced. Exemplary polar plots of the Euclidian distance are presented in Fig. 3B.

Cell migration is an essential biological phenomenon for tissue regeneration, since local or implanted cells need to migrate to the site of tissue repair [10]. In the presented study, cell velocity and Euclidian distance were quantified to evaluate cell migration. Since the surface is of great importance for cell migration, four surface coatings were evaluated in both culture conditions. Hypoxia significantly promoted cell migration on all substrates. Furthermore, the surface had a significant impact on cell velocity, while the increase was more pronounced in normoxic cell culture. Laminin in both oxygen conditions, led to the highest elevation of hMSC velocity [17]. Only five studies evaluated the influence of hypoxia on hMSC migration [5,18–21]. These studies report contradictory results. One possible explanation is that all studies apply varying periods

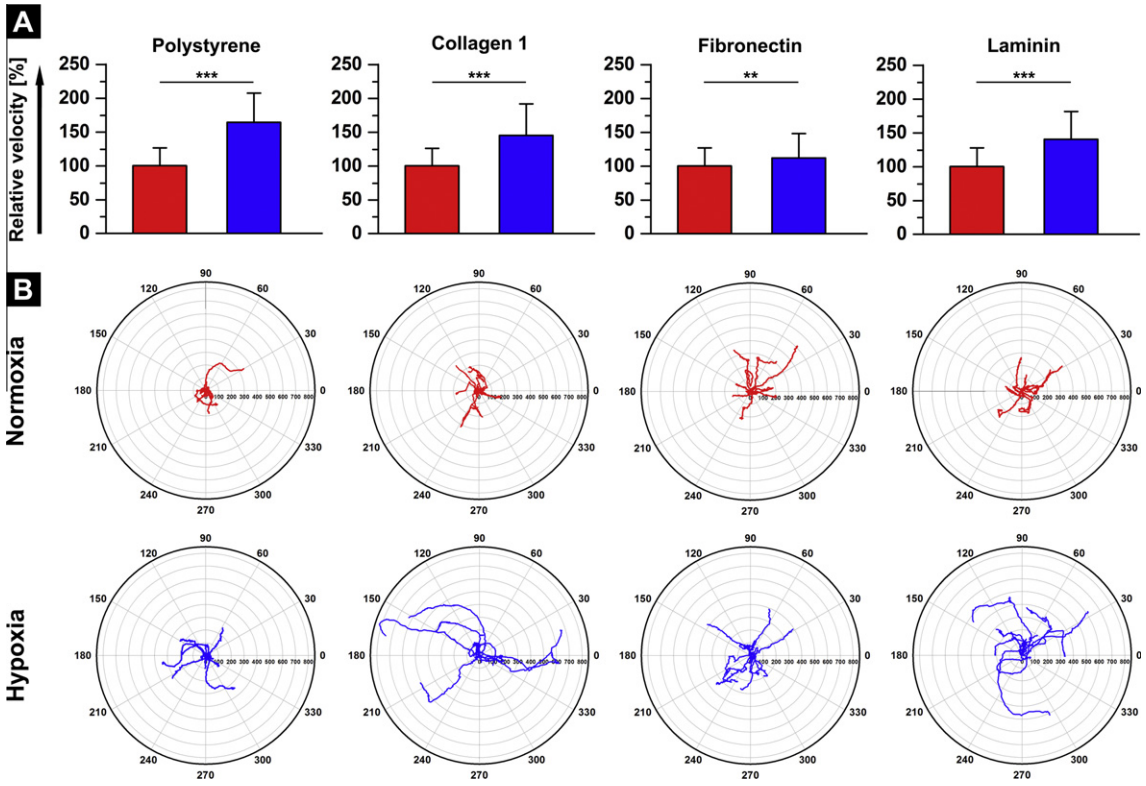

Fig. 3. Cell migration on different ECM proteins. (A) Normoxic cells (red) were set to 100%, blue reflects hypoxia-cultured cells. Results are presented as mean, error bars = SD, ** < 0.01, *** < 0.001. (B) Exemplary cell tracks, polar circles indicate the Euclidian distance [μm], radial lines the movement angle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

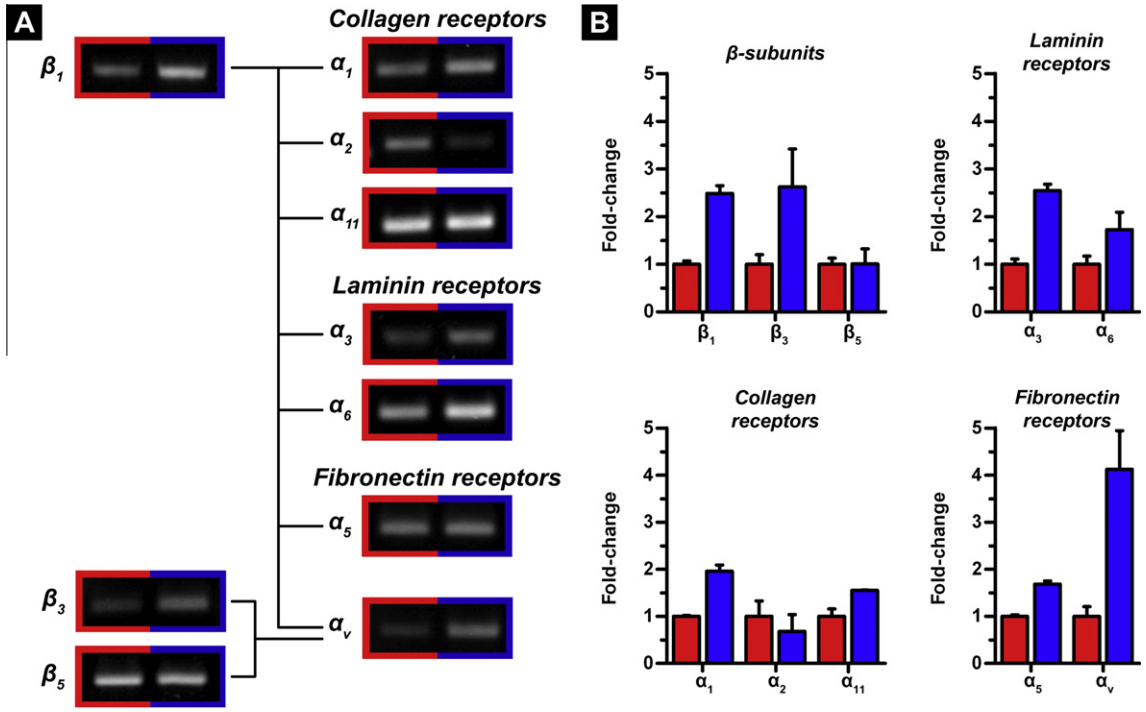

Fig. 4. Comparison of integrin expression between normoxic (red) and hypoxic (blue) culture conditions by RT-PCR. (A) Exemplary RT-PCR results. (B) Quantitative analysis of integrin expression normalised to GAPDH and presented as fold changes to normoxia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of hypoxic preconditioning, meaning cells were cultured in hypoxic conditions for the time of the experiments only or a limited time prior. To our knowledge our study is the only one culturing the cells in hypoxic conditions for the entire culture period, thereby mimicking the conditions in the stem cell niche. Furthermore, all studies used indirect measures (e.g. scratch-assay, wound-healing-assay, boyden chamber) to assess migration. We performed a time-lapse analysis to evaluate cell migration for 72 h by direct assessment of cell movement. Finally, we carried out our analysis on 4 different extracellular matrix proteins typically located in the bone. We therefore conclude that both, the surface and the oxygen concentration have a great effect on hMSC migration. Our results reveal that hMSC migration is increased in hypoxic conditions on various surfaces, though the exact mechanism needs to be further elucidated.

3.5. Integrin expression is altered in hypoxic hMSCs

Cell-matrix and cell–cell interaction is influenced by integrin expression, therefore we screened the integrin expression of the collagen I α-subunits 1,2,11, the fibronectin α-subunits 3 and 6, as well as α_v and α_5 subunits for laminins. The corresponding β-subunits 1, 3 and 5 were also investigated. The expression of integrin α-subunits 1, 3, 5, 6, 11, v and the β-subunits 1 and 3 were augmented in hypoxic hMSCs compared to normoxic. In contrast, α_2 expression was higher in normoxic cultured cells. No differences could be observed for β_5. An overview of all screened integrins is presented in Fig. 4A and densitometric expression analyses are shown in Fig. 4B.

Interestingly, it has been shown, that artificially added reactive oxygen species (ROS) significantly reduce integrin expression of α_v and β_1 on protein level within MSCs [22]. Due to the fact, that hypoxia increases the intracellular ROS levels [22], some focal adhesion complexes might also be degraded in hypoxic conditions. In contrast, our results revealed an upregulation of various integrin subunits in a low oxygen concentration, and therefore a protective mechanism to avoid that degradation must be involved. Busletta et al. [18] showed that hypoxic-dependent migration is induced by mitochondrial ROS release, which activates ERK/JNK-pathways and finally stabilizes HIF-1α. This intracellular ROS release differs from externally added ROS due to an adaptive feedback-loop via vascular endothelial growth factor (VEGF) and subsequent H_2O_2 generation by NADPH-oxidase [18]. This controlled HIF-1α stabilization has been shown to be a major signal for integrin regulation within hypoxia [23]. Furthermore, formation of focal adhesions is involved in the cell migration. The altered integrin expression thereby presumably influences the increased cell migration in hypoxic conditions. Our study served to first identify more effects of hypoxia on hMSC and second to identify possible targets for further knockdown and biomechanical experiments, as such screening experiments have not been performed up to date. Based on our findings we set up a follow up study, currently in progress, using knockdown and biochemical experiments to clarify the exact pathways involved in hypoxia related changes of cell morphology, migration, and integrin expression.

Taken together, our study has demonstrated the fundamental impact of the oxygen concentration on various hMSC characteristics in vitro. HMSCs favour hypoxic conditions in terms of stemness and migration. Furthermore, we could demonstrate that low oxygen concentration alters integrin expression. Future experiments will have to elucidate the exact pathways behind the observed increased migration in hypoxia as well as the exact role of the altered integrin expression.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.134.

References

[1] T. Ma, W.L. Grayson, M. Frohlich, G. Vunjak-Novakovic, Hypoxia and stem cell-based engineering of mesenchymal tissues, Biotechnology progress 25 (2009) 32–42.
[2] A. Augello, T.B. Kurth, C. De Bari, Mesenchymal stem cells: a perspective from in vitro cultures to in vivo migration and niches, European cells & materials 20 (2010) 121–133.
[3] W.L. Grayson, F. Zhao, B. Bunnell, T. Ma, Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells, Biochemical and Biophysical Research Communications 358 (2007) 948–953.
[4] E. Volkmer, B.C. Kallukalam, J. Maertz, S. Otto, I. Drosse, H. Polzer, W. Bocker, M. Stengele, D. Docheva, W. Mutschler, M. Schieker, Hypoxic preconditioning of human mesenchymal stem cells overcomes hypoxia-induced inhibition of osteogenic differentiation, Tissue engineering. Part A 16 (2010) 153–164.
[5] S.P. Hung, J.H. Ho, Y.R. Shih, T. Lo, O.K. Lee, Hypoxia promotes proliferation and osteogenic differentiation potentials of human mesenchymal stem cells, Journal of Orthopaedic Research 30 (2012) 260–266.
[6] H. Ren, Y. Cao, Q. Zhao, J. Li, C. Zhou, L. Liao, M. Jia, H. Cai, Z.C. Han, R. Yang, G. Chen, R.C. Zhao, Proliferation and differentiation of bone marrow stromal cells under hypoxic conditions, Biochemical and Biophysical Research Communications 347 (2006) 12–21.
[7] D.J. Prockop, I. Sekiya, D.C. Colter, Isolation and characterization of rapidly self-renewing stem cells from cultures of human marrow stromal cells, Cytotherapy 3 (2001) 393–396.
[8] F. Haasters, W.C. Prall, D. Anz, C. Bourquin, C. Pautke, S. Endres, W. Mutschler, D. Docheva, M. Schieker, Morphological and immunocytochemical characteristics indicate the yield of early progenitors and represent a quality control for human mesenchymal stem cell culturing, Journal of Anatomy 214 (2009) 759–767.
[9] D. Docheva, D. Padula, M. Schieker, H. Clausen-Schaumann, Effect of collagen I and fibronectin on the adhesion, elasticity and cytoskeletal organization of prostate cancer cells, Biochemical and Biophysical Research Communications 402 (2010) 361–366.
[10] D.A. Lauffenburger, A.F. Horwitz, Cell migration: a physically integrated molecular process, Cell 84 (1996) 359–369.
[11] P. Alberton, C. Popov, M. Pragert, J. Kohler, C. Shukunami, M. Schieker, D. Docheva, Conversion of Human Bone Marrow-Derived Mesenchymal Stem Cells into Tendon Progenitor Cells by Ectopic Expression of Scleraxis, Stem Cells and Development 21 (2012) 846–858.
[12] C. Popov, T. Radic, F. Haasters, W.C. Prall, A. Aszodi, D. Gullberg, M. Schieker, D. Docheva, Integrins alpha2beta1 and alpha11beta1 regulate the survival of mesenchymal stem cells on collagen I, Cell Death & Disease 2 (2011) e186.
[13] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, E. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells, The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317.
[14] T. Mets, G. Verdonk, In vitro aging of human bone marrow derived stromal cells, Mechanisms of Ageing and Development 16 (1981) 81–89.
[15] D.C. Colter, R. Class, C.M. DiGirolamo, D.J. Prockop, Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 3213–3218.
[16] Y.E. Korchev, J. Gorelik, M.J. Lab, E.V. Sviderskaya, C.L. Johnston, C.R. Coombes, I. Vodyanoy, C.R. Edwards, Cell volume measurement using scanning ion conductance microscopy, Biophysical Journal 78 (2000) 451–457.
[17] Y. Rattigan, J.M. Hsu, P.J. Mishra, J. Glod, D. Banerjee, Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu, Experimental Cell Research 316 (2010) 3417–3424.
[18] C. Busletta, E. Novo, L. Valfre Di Bonzo, D. Povero, C. Paternostro, M. Ievolella, K. Mareschi, I. Ferrero, S. Cannito, A. Compagnone, A. Bandino, S. Colombatto, F. Fagioli, M. Parola, Dissection of the biphasic nature of hypoxia-induced

motogenic action in bone marrow-derived human mesenchymal stem cells, Stem Cells 29 (2011) 952–963.
[19] S.H. Lee, Y.J. Lee, H.J. Han, Role of hypoxia-induced fibronectin-integrin beta1 expression in embryonic stem cell proliferation and migration: Involvement of PI3K/Akt and FAK, Journal of Cellular Physiology 226 (2011) 484–493.
[20] L.F. Raheja, D.C. Genetos, A. Wong, C.E. Yellowley, Hypoxic regulation of mesenchymal stem cell migration: the role of RhoA and HIF-1alpha, Cell Biology International 35 (2011) 981–989.
[21] I. Rosova, M. Dao, B. Capoccia, D. Link, J.A. Nolta, Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells, Stem Cells 26 (2008) 2173–2182.
[22] H. Song, M.J. Cha, B.W. Song, I.K. Kim, W. Chang, S. Lim, E.J. Choi, O. Ham, S.Y. Lee, N. Chung, Y. Jang, K.C. Hwang, Reactive oxygen species inhibit adhesion of mesenchymal stem cells implanted into ischemic myocardium via interference of focal adhesion complex, Stem Cells 28 (2010) 555–563.
[23] K.D. Cowden Dahl, S.E. Robertson, V.M. Weaver, M.C. Simon, Hypoxia-inducible factor regulates alphavbeta3 integrin cell surface expression, Molecular Biology of the Cell 16 (2005) 1901–1912.