Intracellular pH Monitoring in Stem Cells During Differentiation Using Fluorescence Microscopy and pH-Sensor SypHer-2

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Among the functional changes accompanying the process of differentiation of stem cells, alterations to the intracellular pH are some of the most important parameters. The concentration of protons in the cytoplasm plays an important role in switching metabolic pathways from oxidative phosphorylation to aerobic glycolysis. Modern fluorescence methods of investigation are currently preferable for the study of pH dynamics. Fluorescence microscopy, in combination with genetically-coded sensors and exogenous markers, allows non-invasive investigation of the functional changes underlying the dynamics of differentiation.

The aim of this study was to investigate the dynamic changes in intracellular pH in mesenchymal stem cells (MSCs) undergoing differentiation in three directions: adipogenic, osteogenic, and chondrogenic using fluorescence microscopy and pH-sensor SypHer-2.

Materials and Methods. Differentiation was induced by incubating the human MSCs in commercial adipogenic or osteogenic or chondrogenic mediums. We used the genetically-coded pH-sensor SypHer-2 as a fluorescent probe. To obtain a temporarily transfected line, MSC-SypHer-2, electroporation of cells was performed with this protein. To convert relative units of pH into absolute units we conducted a calibration of the SypHer-2 pH-sensor. We determined the correlation between the intensities of fluorescence caused in the pH-sensor by illumination at 488 nm and that from illumination at 405 nm (I488/I405) for each value of pH using fluorescence microscopy. In accordance with the calibration curve we defined the absolute pH values in non-differentiated MSCs and in the MSCs undergoing adipogenic, osteogenic or chondrogenic differentiation. The data representing changes of intracellular pH were obtained on days 7, 14, and 21 of differentiation.

Results. In our work, we showed acidification of the intracellular pH during adipogenic, chondrogenic, and osteogenic MSC differentiation. Moreover, this work identified a correlation between changes in the dynamics of intracellular pH and the metabolic status changes of the MSCs that had not been previously described. As the pH in cells undergoing adipogenic differentiation is lower than that in other types of MSC differentiation, this is probably connected with the transfer into the cytosol of the citrate necessary for the biosynthesis of fatty acids and with the conversion of malate into pyruvate. The relatively high pH levels found during osteogenic and chondrogenic differentiation enhance the activity of enzymes needed for catalyzing the oxidation of proline and lysine in collagen, with the participation of ascorbic acid, in particular, proline and lysine hydroxylases.

Presented results provide the basis for further development of effective evaluation methods for characterizing the potential of stem cells, particularly by assessing intracellular pH as an indicator of the status of cell differentiation. In addition, these results can serve the development of an integrated approach to assessing functional changes in the stem cells, reflecting the peculiarities of a particular differentiation in the early stages.

Key words: mesenchymal stem cells; differentiation; intracellular pH; metabolism; SypHer-2; fluorescence microscopy.

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Introduction

Differentiation of stem cells is a process whereby they develop into more specialized cell types, resulting in the loss of some of their alternative development potential. Such differentiation takes place both when developing organisms produce new specialized cells, and when adult organisms need to compensate for lost or damaged cells.

Differentiation can change the size, the shape, and membrane potential of the cell, its metabolic activity and its reactions to extracellular and intracellular signals [1]. With the growing role in regenerative medicine of stem cells, and of mesenchymal stem cells (MSCs) in particular, an in-depth investigation of the structural and functional changes occurring during differentiation is becoming especially important.

Among the functional changes accompanying the process of differentiation, alterations to the intracellular pH (pH_i) are some of the most important parameters.

That pH affects many molecular mechanisms inside and outside cells emphasizes the significance both of homeostasis in maintaining pH and in its potential to influence cellular activity. Proton gradients in cells are maintained by pumps and channels such as the Na^+/H^+, HCO_3^-/Cl^- exchangers as well as by V-shaped H^+ pumps and H channels through the plasma membrane [2]. Active or passive changes in pH influence cells' characteristics such as mobility, enzymatic activity, the cell cycle and apoptosis [3, 4]. Such alteration of cell mobility can be caused by differences in the pH of the medium through its impact on the cytoskeleton [5]. pH also influences the activity of most cell enzymes by affecting the shape and charge distribution of the proteins of which they are composed.

It is known that the pH_i of stem cells is much higher than that of normal differentiated cells, so changes in this index can serve as an indicator of the differentiation process [6]. The pH_i of stem cells is more alkaline compared to differentiated cells of the body, while the extracellular pH (pH_{ex}) of stem cells is more acidic [7]. Evidence is currently being revealed that changes in the pH_i play an important regulatory role in the direction of stem cells differentiation. It is known that the pH_i differs during the differentiation of alternative types of stem cell. Thus, a decrease in pH_i contributes to osteogenic differentiation of mesenchymal human stem cells [6], while high pH_i increases the differentiation of embryonic stem cells (ESCs) [8, 9] and of oligodendrocytes [10].

Extracellular pH can also modify the differentiation activity of MSCs. Kohn et al. [11] reported that a decrease in pH_{ex} decreases the amount of collagen and the activity of alkaline phosphatase. However, Leem et al. [12] presented evidence that an alkaline pH_{ex} lowers the activity of alkaline phosphatase and can delay the differentiation of MSCs.

The concentration of protons in the cytoplasm plays an important role in switching metabolic pathways from oxidative phosphorylation to aerobic glycolysis. It is known that an alkaline pH_i is associated with aerobic glycolysis, while oxidative phosphorylation, in its turn, is associated with an acidic pH_i [13].

However, the metabolic status of MSCs undergoing different types of differentiation is fairly variable, and not every MSC differentiation is accompanied by clear switching of metabolism from glycolysis to oxidative phosphorylation [14, 15]. It is possible that such flexibility of metabolism during differentiation could be associated with changes in pH_i. However, there are currently no complete data sets on the changes in the dynamics of pH_i covering the alternative directions of differentiation.

Modern fluorescence methods of investigation are currently preferable for the study of pH dynamics. Fluorescence microscopy, in combination with genetically-coded sensors and exogenous markers, allows non-invasive investigation of the functional changes underlying the dynamics of differentiation.

Fluorescence microscopy used with genetically-coded sensors has a big advantage for pH investigations. The main benefit of genetically-coded sensors (fluorescent proteins) is the possibility of targeted localization of sensor expression both in the cytosol and in any cell organelle. The technique allows the recording of pH in specific cell compartments [16, 17].

Thus, the main objective of this study was to investigate the dynamic changes in pH in three types of MSCs differentiation and to analyze the correlation of this parameter with the metabolic activity of the MSCs during such differentiation.

Materials and Methods

Cell culture. All investigations were performed on MSCs from pieces of human adipose tissues obtained during abdominal plastic surgery. The patients' informed consent was obtained in accordance with the Institute's approved protocol.

Biopsy samples of the fat tissues were processed within 8 h after the operations. Stromal cells of the fat tissue were isolated using Zuk's method [18] with modifications. The tissue was washed in Hank's saline solution with gentamicin (200 units/ml), cut with scissors and incubated with a 0.1% solution of collagenase type I (Worthington, USA) at 37°C for 90 min with continuous mixing. The enzyme was then inhibited with 10% calf serum (BioloT, Russia). Mature adipocytes were isolated by centrifuging at 300 g for 10 min. The packed cells were washed free of the enzyme in DMEM medium (Sigma, Germany) containing 10% calf serum. The cell suspension was filtered through a nylon filter (pore diameter 100 μm) and centrifuged in a density gradient (Histopaque-1077; Sigma, Germany) at 400 g for 30 min at ambient temperature to obtain fractions of mononuclear cells. The cell suspension was washed from Histopaque-1077 in DMEM medium three times. The cells were cultivated until the first passaging in DMEM medium containing 20%

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calf serum, after which the cultures were transferred to DMEM/F12 medium (1:1 by volume), containing 10% calf medium and then cultivated in this medium. The medium was changed every 3–4 days.

Flow cytometry. The MSCs were tested using a flow cytometer (FACSAria III; BD Biosciences, USA) for the presence of markers typical of MSCs (CD105, CD73, CD90, CD34, CD45, HLA-DR, CD11b, and CD19). The commercial Human MSC Analysis Kit (BD Biosciences, USA) was used for characterization.

Induction of adipogenic, chondrogenic and osteogenic differentiation. For the induction of adipogenic, chondrogenic or osteogenic differentiation we used the following commercial mediums, respectively: MesenCult Adipogenic Differentiation Medium (Human), MesenCult Osteogenic Stimulatory Kit (Human) (STEMCELL Technologies, Canada), Stem MACS Chondro Diff Media (Miltenyi Biotec GmbH, Germany). The medium was changed every 3–4 days for 3 weeks.

The course of each type of differentiation was confirmed by observing changes in cell morphology and specific staining. Adipogenic, chondrogenic, and osteogenic differentiation, respectively, were verified with Oil Red O (Sigma, Germany) staining for neutral fats, with Alizarin Red S (Sigma, Germany) for calcification of extracellular matrixes, and with Alcian Blue (Sigma, Germany) for the presence of acidic polysaccharides such as glycosaminoglycans.

Temporary transfection of MSC with SypHer-2. We used the genetically-coded pH-sensor SypHer-2 as a fluorescent probe. SypHer-2 has one emission peak (516 nm) and two peaks of fluorescence excitement (420 and 500 nm), from the correlation of which the pH in its vicinity can be calculated (ratiometrically) [19].

To obtain a temporarily transfected line, MSC-SypHer-2, electroporation of cells was performed with this protein. Four hundred thousand cells were used for transfection. Before the transfection, the MSCs were collected from Petri dishes using Versene solution (PanEco, Russia) and 0.25% trypsin (PanEco, Russia) followed by centrifugation at 200 g for 5 min. The supernatant was decanted. 100 µl of Human MSC Nucleofector Solution (Lonza, USA) was added to the cells and they were re-suspended thoroughly. Then 100 µl of the cell suspension was mixed with 2 ng of plasmid vector SypHer-2. The resulting sample was placed into a special cuvette into which the Nucleofector (Lonza, USA) electroporation device was placed. For high efficiency of transfection, Mode U-23 was chosen. The transfected MSC-SypHer-2 cells were cultivated on 35 mm glass-bottomed dishes.

The temporary SypHer-2 protein transfections of MSCs contained in standard growth medium with additional differentiation medium of the appropriate type were performed 1 day before imaging (i.e. on days 6, 13, and 20 of differentiation). The data representing changes of pH_i were obtained using laser scanning microscopy on days the 7, 14, and 21 of differentiation.

Calibration. To convert relative units of pH into absolute units we conducted a calibration of the SypHer-2 pH-sensor. For this we prepared calibration solutions (130 mM of potassium gluconate, 20 mM of sodium gluconate, 2 mM of $CaCl_2$, 1 mM of $MgCl_2$, 30 mM of HEPES (pH 6.9–7.9), 30 mM of MES (pH 6.0–6.9), 30 mM of Tris (pH 7.9–9.0), and 30 mM of MOPS (pH 6.9–8.0)) to provide pH values of 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.0. MSC-SypHer-2 cultures were placed into the calibration solutions with 10–50 µM of nigericin. Images were recorded after 5 min. From the resulting data we constructed a calibration curve indicating the correlation of fluorescence intensity to pH. The calibration curve was described by the exponential equation: $y = 2E{-}05e^{1.5987x}$.

Fluorescence microscopy. To obtain images we used an LSM 880 (Carl Zeiss, Germany) microscope with a C Plan Apochromat (Carl Zeiss, Germany) oil immersion objective with 40-fold magnification and a numerical aperture of 1.3.

Before imaging, the cells were placed into FluoroBrite medium without phenol red. For the genetically-coded pH-sensor SypHer-2 its two absorption peaks were excited using a diode laser at a wavelength of 405 nm and an argon laser at a wavelength of 488 nm, with the consequent fluorescence being detected in the range 500–550 nm. The resulting images were processed with Image J software (NIH, USA). We then determined the correlation between the intensities of fluorescence caused in the pH-sensor by illumination at 488 nm and that from illumination at 405 nm (I488/I405) for each value of pH. In accordance with the calibration curve we defined the absolute pH values in non-differentiated MSCs and in the MSCs undergoing adipogenic, osteogenic or chondrogenic differentiation.

Statistical analysis. To study pH at each time point of differentiation we analyzed from 7 to 12 cells and from 40 to 58 areas of interest. The statistical analysis was performed in Exel and Statistica 64, version 10 (StatSoft Inc., USA). To present the findings we used mean and their standard deviations (SD). Differences in the mean values were tested for significance using the Student's t-test or the one-way ANOVA with Fisher's post-hoc test ($p \leq 0.05$).

Results

Cell culture and differentiation. With the use of flow cytometry we showed that the MSCs had the phenotype of normal mesenchymal cells and expressed the typical markers: more than 77.5% of cells expressed CD105, CD90, and CD73 while less than 22% expressed the negative surface markers CD34, CD45, HLA-DR, CD11b or CD19 (Figure 1). We confirmed that the MSCs had differentiated in three different directions by staining with specific dyes (Figure 2). On cultivation in induction media the rate of cell proliferation decreased, the cells changed their morphology: increasing in size
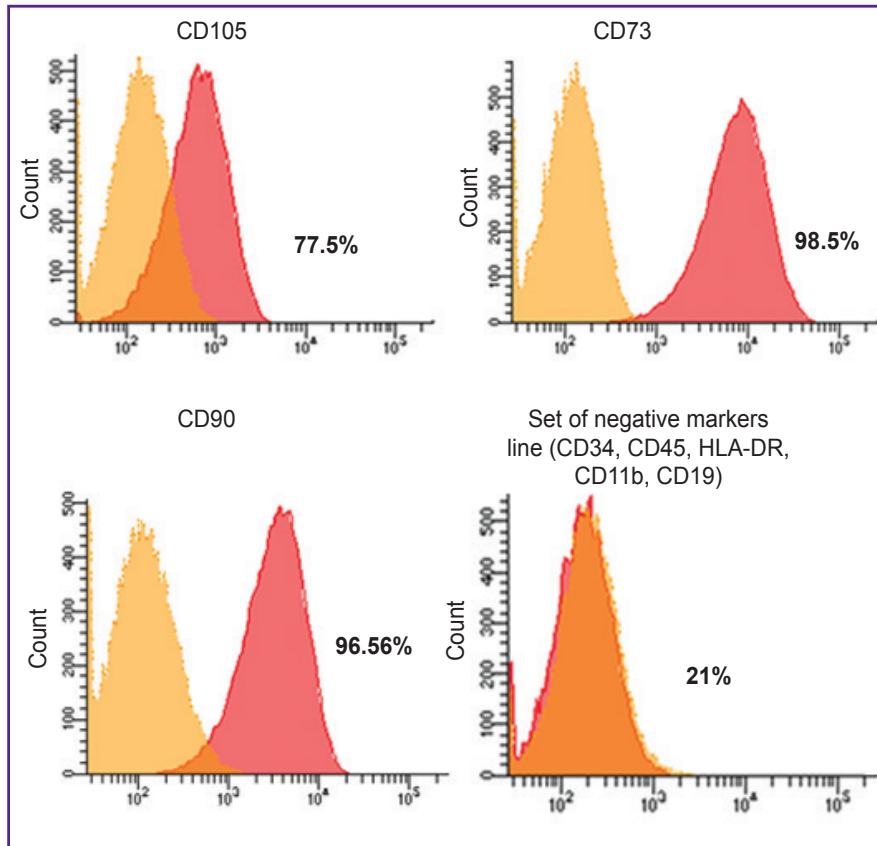
Figure 1. Flow cytometry
Characteristics of mesenchymal stem cells in the main markers: positive markers expression — CD105, CD90, CD73; negative markers expression — CD34, CD45, HLA-DR, CD11b, CD19
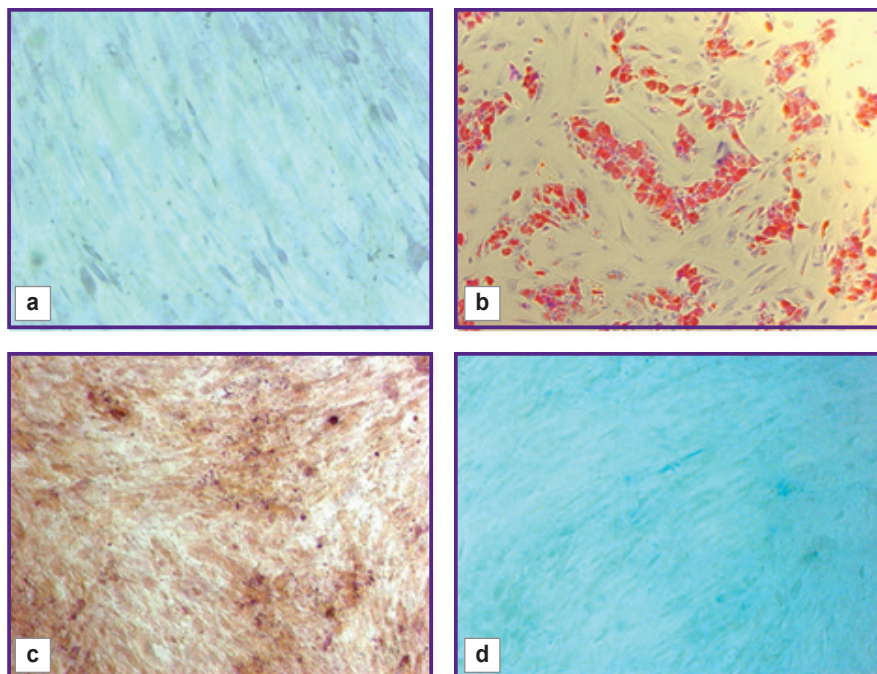

Figure 2. Microscopic images of undifferentiated and differentiated mesenchymal stem cells in transmitted light:
(a) undifferentiated MSCs, incubated in standard growth medium; (b) MSCs on day 21 of adipogenic differentiation (Oil Red O staining); (c) MSCs on day 21 of osteogenic differentiation (Alizarin Red S staining); (d) MSCs on day 21 of chondrogenic differentiation (Alcian Blue staining). The image size is 1289×964 μm

and changing from fusiform to polygonal. With Oil Red O stain we showed that in the cells undergoing adipogenic differentiation, the cytoplasm accumulated neutral fats. Staining with Alizarin Red S indicated calcification of a dense extracellular matrix in the MSCs cultivated in osteogenic medium. In the cells undergoing chondrogenic differentiation, Alcian Blue stain showed the presence of acidic polysaccharides such as glycosaminoglycans.

Temporary transfection with SypHer-2. Constructing the calibration curve. In preliminary experiments we conducted calibration with the pH-sensor SypHer-2 to convert relative pH units into absolute units. From our observations we constructed a calibration curve representing the correlation between fluorescence intensity and pH. This calibration curve was described with an exponential relation equation (Figure 3).
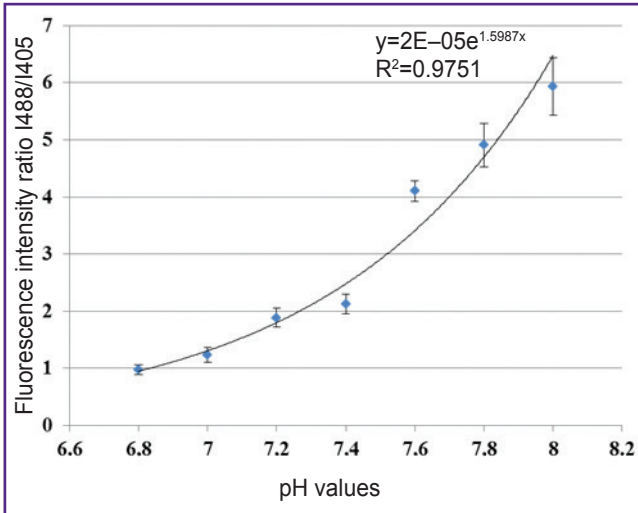
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Figure 3. The calibration dependence curve of the fluorescence intensity ratio from pH values for SypHer-2

Analysis of the intracellular pH levels during adipogenic, chondrogenic, and osteogenic differentiation. To evaluate the levels of pH$_i$ in non-differentiated MSCs and MSCs undergoing differentiation, the cells were successfully transfected with SypHer-2 protein.

The level of pH$_i$ in non-differentiated MSCs was stable for the 3 weeks allowed for differentiation. The average values of correlation of fluorescence intensity in non-differentiated MSCs on weeks 1, 2, and 3 were 4.962±0.463, 4.859±0.810, and 4.761±0.454 relative units, which on the calibration curve corresponded to the absolute values of pH: 7.7±0.08, 7.75±0.15, and 7.74±0.08, respectively.

We observed acidification of pH$_i$ in all three directions of differentiation. The absolute pH$_i$ values on days 7, 14, and 21 of adipogenic differentiation were 7.44±0.09, 7.19±0.1, and 7.19±0.1, respectively (statistically significant differences from non-differentiated MSCs was seen in all the lines of differentiation) (Figures 4, 5).
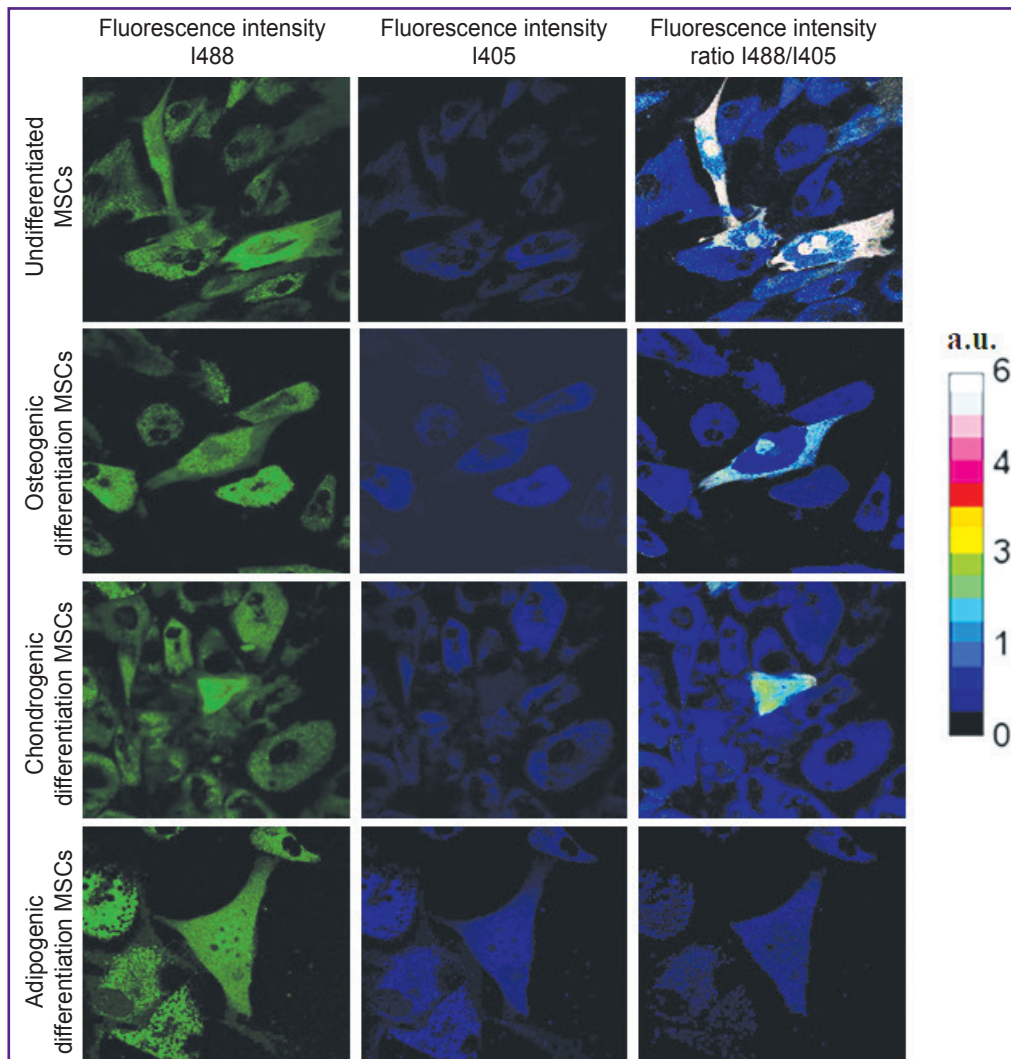

Figure 4. Fluorescence and fluorescence intensity ratio images of undifferentiated MSCs-SypHer-2 and MSCs-SypHer-2 during adipogenic, osteogenic, and chondrogenic differentiation on day 21
Fluorescence excitation of SypHer-2 at 488 and 405 nm, fluorescence detection — 500–550 nm. The image size is 213×213 μm
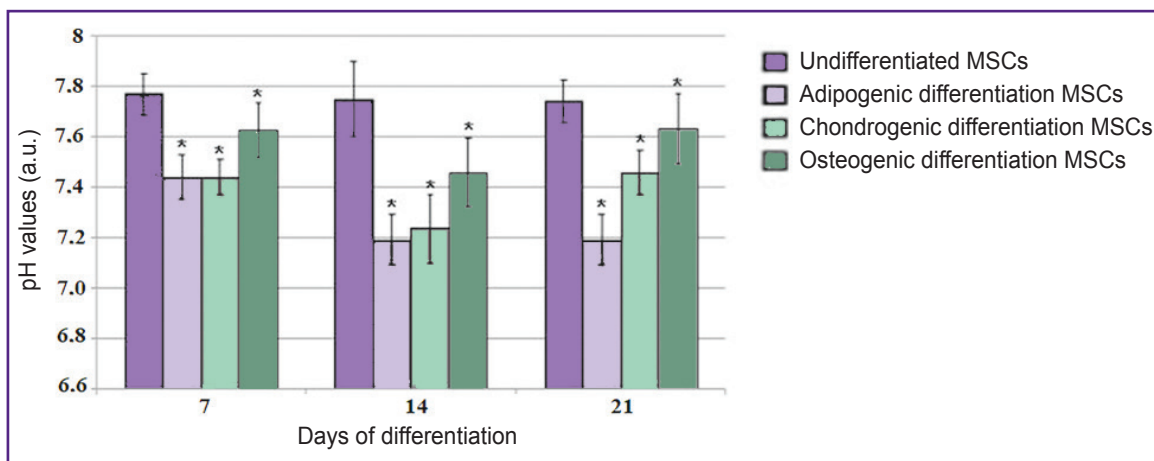
Figure 5. Dynamics of the pH values in undifferentiated MSCs-SypHer-2 and MSCs-SypHer-2 during adipogenic, osteogenic, and chondrogenic differentiation; mean ± SD
*Statistically significant difference with undifferentiated MSCs on the same day, p≤0.00000

During osteogenic differentiation, all the differentiating cells that we observed showed a statistically significant decrease in absolute pH_i compared to those in non-differentiated cells at the same time point (pH_i in differentiating cells: 7.6±0.07, 7.4±0.14, and 7.6±0.09) (see Figures 4, 5).

In the case of chondrogenic differentiation, we also detected a shift toward more acidic pH_i at all the key points of observation (7.4±0.1, 7.2±0.13, and 7.4±0.13). The difference in pH_i was statistically significant compared with non-differentiated MSCs in all the lines of differentiation (see Figures 4, 5).

Discussion

In this study we investigated dynamic changes in pH_i in three targeted types of differentiation of MSCs, and also performed an analysis of the correlation between this parameter and the metabolic activity of the MSCs during such differentiation.

It is known that changes in pH_i regulate the direction of various types of stem cell differentiation. Thus, a decrease in pH_i induced by cariporide (an inhibitor of the pH_i regulator NHE1) contributes to osteogenic differentiation of human MSCs [6]. On the other hand, in cultures of mouse ESCs high pH_i increases differentiation in heart cells [8]. Ulmschneider et al. also showed that during the differentiation of ESCs the pH_i increases from 7.4 to 7.65 [9], while Boussouf et al. [10] identified lower values of pH_i in a primary culture of oligodendrocytes compared to mature oligodendrocytes.

In our work fluorescence microscopy, used with the pH-sensor SypHer-2, showed acidification of the pH_i during adipogenic, chondrogenic, and osteogenic MSC differentiation, results that correlate with the findings of several other authors.

Moreover, this work identified a correlation between changes in the dynamics of pH_i and the metabolic status changes of the MSCs that had not been previously described.

It is fact, that the concentration of protons in the cytoplasm (pH_i) plays a key role in switching metabolic pathways from oxidative phosphorylation to aerobic glycolysis [13].

Alkaline pH accompanies aerobic glycolysis, while oxidative phosphorylation is associated with acidic pHs. The outer mitochondrial membrane contains transport proteins called voltage-dependent anion channels, or mitochondrial porins, which form large channels through the lipid bilayer [20, 21]. When the voltage-dependent anion channels are open, the intermembrane space and cytosol are supposedly chemically equivalent in terms of the small molecules that they contain, including H^+. A decrease in H^+ concentration in the cytosol (intracellular alkalization) therefore decreases the concentration of H^+ in the intermembrane space of the mitochondria. In its turn, this reduces the electrochemical gradient of H^+ across the inner mitochondrial membrane and suppresses oxidative phosphorylation even when there is a sufficient level of oxygen. In addition to this suppression of oxidative phosphorylation, a decrease in the cytosolic concentration of H^+ can stimulate glycolysis. It has been established that the key glycolytic enzyme, phosphofructokinase (PFK), is suppressed by adenosine triphosphate (ATP) [22]. Oxidative phosphorylation repression induced by intracellular alkalization decreases the level of cellular ATP; this leads to the inhibition of PFK by ATP and thus increases the rate of glycolysis to compensate for the ATP deficiency. Furthermore, our data testify to the fact that intracellular alkalization activates glycolysis by a direct increase in PFK activity. The PFK enzyme molecule is extremely sensitive to small changes

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in pH in the physiological range, high pHs increasing its activity. Specifically, a pH increase of 0.1–0.3 can change the activity of PFK from its inactive form to its saturated state [23, 24].

As a decrease in the cytosolic concentration of H$^+$ (intracellular alkalization) can lead to metabolic switching from oxidative phosphorylation to aerobic glycolysis, it can be expected that an increase in H$^+$ levels in cytosol (intracellular acidification) would reverse this metabolic switching. It is well-known that activation of glycolysis produces H$^+$ in the cytosol. This is why cells revert from aerobic glycolysis to oxidative phosphorylation, when the activation of glycolysis leads to a concentration of H$^+$ that is high enough to reverse intracellular alkalization. This increase in the cytosol concentration of H$^+$ increases the electrochemical gradient of H$^+$ across the inner mitochondrial membrane, allowing the penetration of pyruvates and non-organic phosphates (Pi) into the matrix, and activates oxidative phosphorylation. This reduction of pH$_i$ will also inhibit glycolysis by direct and indirect inhibition of PFK activity and by reduction of the expression of glucose transporters and glycolytic enzymes induced by pyruvate and mediated by HIF-1 [25, 26].

The switching of metabolic pathways is a marker of the transition of stem cells from their undifferentiated status to a differentiated state. As a rule, undifferentiated stem cells use glycolysis as the fastest way of obtaining energy, while differentiated cells, by contrast, switch to oxidative phosphorylation [27]. Stem cells proliferate actively and require greater energy expenditure for the synthesis of macromolecules than do normal differentiated cells. Due to their greater need to synthesize the required compounds, glycolysis prevails in highly-proliferating cells as the fastest method of ATP synthesis. In differentiated cells oxidative phosphorylation prevails. However, not every case of differentiation illustrates clear switching of metabolic pathways.

Taking the above into account, it is clear that flexible metabolism during stem cell differentiation must be accompanied by corresponding shifts in pH$_i$.

Actually, on the basis of our analysis, we were able to identify correlations between the changes in pH$_i$ and the changes in MSC metabolic status during the three types of differentiation.

Our previous work, based on changes in the lifetime of NADPH and FAD fluorescence, has demonstrated the switching of metabolism from glycolysis to oxidative phosphorylation in adipogenic differentiation, from glycolysis to oxidative phosphorylation and back to glycolysis in osteogenic differentiation, and from glycolysis to a more glycolytic status in chondrogenic differentiation [28–30].

In adipogenic MSC differentiation, obvious changes in metabolism (transition from glycolysis to oxidative phosphorylation) were registered in week 3 [28, 29]. This was indicated by the increased contributions of the fluorescence lifetime of bound NADPH (up to 12%), bound NADH (34.5%), and free FAD (33%), which

perhaps indicate enhancement of stages 6–10 of the Krebs cycle (higher impact of oxidative phosphorylation) and of fatty acids biosynthesis. There was a lesser impact on bound FAD in adipogenic differentiation compared to non-differentiated MSCs (66% against 76%, respectively) allowing us to suppose a decrease in the emphasis on fatty acid β-oxidation and the work of the glycerol-3-phosphate shuttle system.

In the case of pH changes during differentiation, we observed a consistent decrease in the values at all stages of the differentiation process compared to undifferentiated MSCs. Low pH values (7.2) in week 3 of differentiation, along with relatively high proportions of free FAD (34%) also support the idea of lowered activity of the M-A shuttle system due to enhancement of stages 6–10 of the Krebs cycle, as well as of the glycerol-3-phosphate shuttle system, due to the use of glycerol for triglyceride biosynthesis, the presence of which is confirmed by the formation of lipid vacuoles during that week. Perhaps, these low pH values can lead to a decrease contribution of free NADH and this may result in lower emphasis on glycolysis, the activity of which is known to fall when the pH falls.

In osteogenic differentiation, especially in week 1, we observed higher values of bound NADH (40%) and of free FAD (40%), indicating relatively high activity of the Krebs cycle in the cells. Beginning from week 2 there was a relative decrease in bound NADH (to as much as 30%) and of free FAD (32%), these values indicating a decrease in Krebs cycle activity [30]. The decline in free FAD gives us grounds to suppose there is some enhancement of fatty acid biosynthesis. This is confirmed by the presence of individual fat droplets.

At the same time, the relatively high values of bound FAD and free NADH allow us to suggest that these cells preserve fairly high levels of β-oxidation of fatty acids, supplying substrates for oxidative phosphorylation, resulting in a resumption of ATP generation, which is necessary for the biopolymer biosynthesis pathways.

Throughout the whole period of osteogenic MSC differentiation relatively high pH values (at least 7.4) were maintained. The pH reduction in week 2 probably reflects metabolic changes in the direction of enhancement of biopolymer biosynthesis (collagen) and the creation of triglyceride reserves (formation of fat droplets) [29]. However, the high metabolic rate in the cells provides coordination between the processes of formation, transfer, and disposal of the reconstitution equivalents and the processes of biopolymer biosynthesis, together with the greater buffering capacity of the cells' buffer systems during this differentiation.

In chondrogenic differentiation, the high levels of free NADH in the cells, obviously reflect the high rate of glycolysis in these cells at all stages of differentiation [30]. Although cells undergoing chondrogenic differentiation had lower metabolic rates than the undifferentiated MSCs and osteogenically

differentiating cells during the whole observation period, they were likely to have shown both greater β-oxidation of fatty acids and greater emphasis on glycolysis (high values of bound FAD). Moreover, as in osteogenic differentiation, in chondrogenically differentiating MSCs, the relatively high pH values (not less than 7.4) were maintained for the whole three weeks. A decrease in pH (to 7.2) during week 2 in the cells of this line may possibly be connected with some decoupling of the transfer from cytosol to mitochondria and the disposal of reconstitution equivalents, as well as to the biosynthesis of high-molecular-mass proteins (collagen) and carbohydrates in the cytosol.

The fact that pH in cells undergoing adipogenic differentiation is lower than that in other types of MSC differentiation is probably connected with the transfer of the citrate, necessary for the biosynthesis of fatty acids, into the cytosol and with the conversion of malate into pyruvate.

The relatively high pH levels found during osteogenic and chondrogenic differentiation enhance the activity of enzymes needed for catalyzing the oxidation of proline and lysine in collagen, with the participation of ascorbic acid, in particular, proline and lysine hydroxylases. For this α-ketoglutarate and a pH of around 7.6 are also needed.

Conclusion

Presented results provide the basis for further development of effective evaluation methods for characterizing the potential of stem cells, particularly by assessing intracellular pH as an indicator of the status of cell differentiation. In addition, these results can serve the development of an integrated approach to assessing functional changes in the stem cells, reflecting the peculiarities of a particular differentiation in the early stages.

Competing interests. The authors declare that they have no competing interests.

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