Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Functional expression of adrenoreceptors in mesenchymal stromal cells derived from the human adipose tissue

Polina D. Kotova [a], Veronika Yu. Sysoeva [b], Olga A. Rogachevskaja [a], Marina F. Bystrova [a], Alisa S. Kolesnikova [a], Pyotr A. Tyurin-Kuzmin [b], Julia I. Fadeeva [b], Vsevolod A. Tkachuk [b], Stanislav S. Kolesnikov [a,*]

[a] Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia
[b] Department of Biochemistry and Molecular Medicine, Faculty of Basic Medicine, Lomonosov Moscow State University, Russia

ARTICLE INFO

Article history:
Received 1 March 2014
Received in revised form 1 May 2014
Accepted 9 May 2014
Available online xxxx

Keywords:
Mesenchymal stromal cell
Adrenergic receptor
Calcium-induced calcium release
Ca^{2+} uncaging
IP_3 receptor

ABSTRACT

Cultured mesenchymal stromal cells (MSCs) from different sources represent a heterogeneous population of proliferating non-differentiated cells that contains multipotent stem cells capable of originating a variety of mesenchymal cell lineages. Despite tremendous progress in MSC biology spurred by their therapeutic potential, current knowledge on receptor and signaling systems of MSCs is mediocre. Here we isolated MSCs from the human adipose tissue and assayed their responsivity to GPCR agonists with Ca^{2+} imaging. As a whole, a MSC population exhibited functional heterogeneity. Although a variety of first messengers was capable of stimulating Ca^{2+} signaling in MSCs, only a relatively small group of cells was specifically responsive to the particular GPCR agonist, including noradrenaline. RT-PCR and immunocytochemistry revealed expression of α1B-, α2A-, and β2-adrenoreceptors in MSCs. Their sensitivity to subtype-specific adrenergic agonists/antagonists and certain inhibitors of Ca^{2+} signaling indicated that largely the α2A-isoform coupled to PLC endowed MSCs with sensitivity to noradrenaline. The all-or-nothing dose-dependence was characteristic of responsivity of robust adrenergic MSCs. Noradrenaline never elicited small or intermediate responses but initiated large and quite similar Ca^{2+} transients at all concentrations above the threshold. The inhibitory analysis and Ca^{2+} uncaging implicated Ca^{2+}-induced Ca^{2+} release (CICR) in shaping Ca^{2+} signals elicited by noradrenaline. Evidence favored IP_3 receptors as predominantly responsible for CICR. Based on the overall findings, we inferred that adrenergic transduction in MSCs includes two fundamentally different stages: noradrenaline initially triggers a local and relatively small Ca^{2+} signal, which next stimulates CICR, thereby being converted into a global Ca^{2+} signal.

1. Introduction

Apart from fully differentiated cells, apparently all postnatal mammalian tissues contain rare and quiescent stem cells that have the capacity for self-renewal and initiating differentiated descendants [1,2]. Mesenchymal stem cells are an uncommon population present in the bone marrow and tissues, such as the adipose tissue, synovium, dermis, periosteum, and deciduous teeth. During isolation and expansion in vitro mesenchymal stem cells constitute a minor fraction (<1%) of the total mesenchymal stromal cell (MSC) population. The increasing body of evidence points to the intrinsic, and donor-to-donor heterogeneity of MSC populations [3–5]. One of the pioneering demonstrations of populational diversity was provided by the microSAGE assay of MSC colonies derived from a single human bone marrow cell. The expression analysis revealed transcripts for more than 2000 genes encoding proteins known to regulate angiogenesis, cell motility, hematopoiesis, immunity, and neural activities [6,7]. It seems to be highly unlikely if an individual cell would be capable of fulfilling all these autonomous physiological functions. Consistently, immunostaining data suggested a non-uniform pattern of protein expression [7]. It was therefore postulated that a variety of functional attributes characteristic of the entire MSC population was determined by separate subpopulations. The concept of intrinsic heterogeneity of a MSC population is well consistent with the finding that a MSC colony can release a broad array of signaling molecules, including FGF-2, BDNF, IGF1, ANG1 and certain cytokines and chemokines [8]. Although mammalian MSCs are poorly characterized in terms of intracellular signaling and ionic mechanisms of electrogenesis, available reports also evidence for molecular and functional heterogeneity of MSC populations [9,10].

Several key factors determine cell proliferation and differentiation, including intrinsic genetic programs, intracellular signaling, and local environmental cues. Reportedly, spontaneous Ca^{2+} oscillations and induced Ca^{2+} transients can mediate development, from fertilization through proliferation and differentiation to organogenesis, and expansion of progenitor pools [11–13]. Thus, membrane receptors and regulatory

* Corresponding author at: Institute of Cell Biophysics, Russian Academy of Sciences, Institutional Street 3, Pushchino, Moscow Region 142290, Russia. Tel.: +7 96 773 9121; fax: +7 96 733 0509.
E-mail address: staskolesnikov@yahoo.com (S.S. Kolesnikov).

http://dx.doi.org/10.1016/j.bbamcr.2014.05.002
0167-4889/

pathways associated with intracellular Ca^{2+} signaling can be critically important for tissue development and regeneration. Although a role of ion channels is much less obvious, they may also be involved in coordinating extracellular and intracellular signals associated with cell proliferation and differentiation [10,14–16]. Specifically, ion channels can regulate exocytosis of autocrine and paracrine factors by determining membrane potential and Ca^{2+} influx [16]. Transmembrane ion fluxes drive cell volume oscillations in cycling and migrating cells [17,18]. Yet, ion channels are capable of modulating cell proliferation and differentiation via flux-independent mechanisms, including association of channel proteins with membrane receptors and related proteins [19–22].

In light of molecular and functional heterogeneity of a MSC population posed by the abovementioned facts, the important possibility is that molecular and functional phenotypes of these cells may be in strong correlation. If so, an individual MSC subpopulation with a specific molecular profile could be revealed physiologically and described in terms of responsivity to agonists, Ca^{2+} signaling pathways, and/or activity of ion channels. We therefore attempted to characterize responsiveness of adipose-derived human MSCs to a variety of agonists, such as ACh, ATP, noradrenaline, serotonin, and some others, which are known to mobilize cytosolic Ca^{2+} in cells of diverse types. All first messengers probed by us were found to stimulate Ca^{2+} signaling in MSCs. Since apart from ATP, a given cell usually responded to one of them, it appears that different MSC subpopulations can indeed be classified based on functional criteria. Here we focused largely on the analysis of MSCs responsive to noradrenaline, given some interesting aspects of Ca^{2+} signaling in adrenergic cells.

2. Materials and methods

2.1. Cell isolation and culturing

Human MSCs were isolated from abdominal subcutaneous adipose tissue harvested during surgical operations from patients at age within 32–60 years. All donors gave informed consent for harvesting their adipose tissue. Donors with infectious or systemic diseases and malignancies were not included in the study. Adipose tissue was extensively washed with 2 volumes of Hank's Balanced Salt Solution (HBSS) containing 1% antibiotic/antimycotic solution (HyClone) and then digested at 37 °C for 1 h in the presence of collagenase (66.7 U/ml, Sigma-Aldrich) and dispase (10 U/ml, BD Biosciences). Enzymatic activity was neutralized by adding an equal volume of culture medium (AdvanceStem basal medium for human undifferentiated mesenchymal stem cells (HyClone) containing 10% of Advance stem cell growth supplement (CGS) (HyClone), 1% antibiotic/antimycotic solution) and centrifuged at 200 *g* for 10 min. This led to sedimentation of diverse cells, including MSCs, macrophages, lymphocytes, and erythrocytes, while mature adipocytes remained floating. After removal of supernatant, a lysis solution (154 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA) was added to a cell pellet to lyse erythrocytes, and cell suspension was centrifuged at 200 *g* for 10 min. Sedimented cells were resuspended in the MSC culture medium and filtered through a 100 μm nylon cell strainer (BD Biosciences). As indicated by flow cytometry data (Supplementary Materials, Fig. 1S), after isolation and overnight pre-plating, the obtained cell population contained largely not only MSC cells but also macrophages and lymphocytes. The two last cell groups were dramatically depleted by culturing during 1 week in the MSC culture medium and humidified atmosphere (5% CO_2) at 37 °C. The obtained MSC population was maintained at a sub-confluent level (<80% confluency) and passaged using HyQTase (HyClone). For experiments, cells of the second to fourth passages were used.

2.2. Preparation of cells for Ca^{2+} imaging

Before assaying with Ca^{2+} imaging, cells were maintained in a 12-socket plate for 12 h in the medium described above but without antibiotics. For isolation, cells cultured in a 1 ml socket were rinsed twice with the Versen solution (Sigma-Aldrich) that was then substituted for 0.2 ml HyQTase solution (HyClone) for 3–5 min. The enzymatic treatment was terminated by the addition of a 0.8 ml culture medium to a socket. Next, cells were resuspended, put into a tube, and centrifuged at 1.2 *g* for 45 s for sedimentation. Isolated cells were collected by a plastic pipette and plated onto a recording chamber of nearly 150 μl volume. The last was a coverslip (Menzel-Glaser) with attached ellipsoidal resin wall. The chamber bottom was coated with Cell-Tak (BD Biosciences) allowing for strong cell adhesion. Attached cells were then loaded with dyes at room temperature (23–25 °C) by adding Fluo-4AM (4 μM) or Fluo-4AM (4 μM) + NP-EGTA-AM (4 μM) and Pluronic (0.02%) (all from Molecular Probes) to the bath solution (mM): NaCl — 110, KCl — 5.5, $CaCl_2$ — 2, $MgSO_4$ — 0.8, NaH_2PO_4 — 1, HEPES — 10, and glucose — 10, pH 7.4. After 20-min incubation cells were rinsed several times with the bath solution and stored at 4 °C for 40 min. When necessary, 2 mM $CaCl_2$ in the bath solution was replaced with 0.5 mM EGTA + 0.4 mM $CaCl_2$, thus reducing free Ca^{2+} to nearly 260 nM.

2.3. Ca^{2+} imaging and uncaging

Most of experiments were carried out using an inverted fluorescent microscope Axiovert 135 equipped with an objective Plan NeoFluar 20x/0.75 (Zeiss) and a digital ECCD camera LucaR (Andor Technology). Apart from a transparent light illuminator, the microscope was equipped with a hand-made system for epi-illumination via an objective. The epi-illumination was performed using a bifurcational glass fiber. One channel, which was used for Fluo-4 excitation, transmitted 480 ± 5 nm irradiation emitted by LED controlled by a computer. Fluo-4 emission was collected at 535 ± 20 nm. Serial fluorescent images were captured every second and analyzed using Imaging Workbench 6 software (INDEC). Deviations of cytosolic Ca^{2+} from the resting level were quantified by a relative change in the intensity of Fluo-4 fluorescence ($\Delta F/F_0$) recorded from an individual cell. Another channel was connected to a pulsed solid laser DTL-374QT (30 mW) (Laser-Export, Moscow). This unit operated in a two-harmonic mode and generated not only 355 nM UV light used for Ca^{2+} uncaging but also visible light at 532 nm. The last could penetrate into an emission channel through non-ideal optical filters and elicited optical artifacts during uncaging. For Ca^{2+} uncaging, cells were loaded with both 4 μM Fluo-4 and NP-EGTA (both from Invitrogen). Other compounds were from Sigma-Aldrich or Tocris.

2.4. Immunofluorescent analysis

For immunofluorescence analysis cultured cells were washed with PBS and fixed in 4% formaldehyde for 4 min at room temperature. Cells were then washed with PBS. Nonspecific binding was blocked by incubation in 1% BSA with 10% serum of secondary antibody donor for 30 min. Immunostaining was performed using mouse antibody against human α1-adrenoreceptor (Abbiotec) and rabbit polyclonal antibody against the human α2-adrenoreceptor (Thermo Sci). Cells stained with primary antibodies were then incubated in the presence of secondary antibodies, Alexa goat anti-rabbit 488 (Molecular Probes, Carlsbad, CA) or Alexa goat anti-mouse 488 (Molecular Probes). Cells were counterstained with nuclear 4,6-diamidino-2-phenylindole (Molecular Probes). As a negative control, cells were treated with mouse or rabbit nonspecific IgGs at relevant concentrations. Immunofluorescence was captured using a Leica DM6000 microscope equipped with a Leica DFC360 FX camera and Las AF software (Leica).

2.5. RT-PCR

Total RNA was extracted from a sample containing 10^5–10^6 MSCs by using the RNeasy mini kit (Qiagen). Isolated RNA was treated with

Please cite this article as: P.D. Kotova, et al., Functional expression of adrenoreceptors in mesenchymal stromal cells derived from the human adipose tissue, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbamcr.2014.05.002