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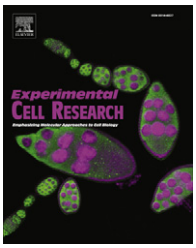

Research Article

Platelet lysate suppresses the expression of lipocalin-type prostaglandin D2 synthase that positively controls adipogenic differentiation of human mesenchymal stromal cells

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ABSTRACT

Mesenchymal stromal cells (MSCs) have been shown to display a considerable therapeutic potential in cellular therapies. However, harmful adipogenic maldifferentiation of transplanted MSCs may seriously threaten the success of this therapeutic approach. We have previously demonstrated that using platelet lysate (PL) instead of widely used fetal calf serum (FCS) diminished lipid accumulation in adipogenically stimulated human MSCs and identified, among others, lipocalin-type prostaglandin D2 synthase (L-PGDS) as a gene suppressed in PL-supplemented MSCs. Here, we investigated the role of PL and putatively pro-adipogenic L-PGDS in human MSC adipogenesis.

Next to strongly reduced levels of L-PGDS we show that PL-supplemented MSCs display markedly decreased expression of adipogenic master regulators and differentiation markers, both before and after induction of adipocyte differentiation. The low adipogenic differentiation capability of PL-supplemented MSCs could be partially restored by exogenous addition of L-PGDS protein. Conversely, siRNA-mediated downregulation of L-PGDS in FCS-supplemented MSCs profoundly reduced adipocyte differentiation. In contrast, inhibiting endogenous prostaglandin synthesis by aspirin did not reduce differentiation, suggesting that a mechanism such as lipid shuttling but not the prostaglandin D2 synthase activity of L-PGDS is critical for adipogenesis.

Our data demonstrate that L-PGDS is a novel pro-adipogenic factor in human MSCs which might be of relevance in adipocyte metabolism and disease. L-PGDS gene expression is a potential quality marker for human MSCs, as it might predict unwanted adipogenic differentiation after MSC transplantation.

Abbreviations: L-PGDS, Lipocalin-type prostaglandin D2 synthase; MSCs, Mesenchymal stromal cells; PL, Platelet lysate; FCS, Fetal calf serum; PPARγ, Peroxisome proliferator-activated receptor gamma; CEBPα, CCAAT/enhancer binding protein alpha; FABP4, Fatty acid binding protein 4; PLIN, Perilipin; GLUT4, Glucose transporter member 4; APOE, Apolipoprotein E; SREBP1, Sterol regulatory element-binding transcription factor 1; LXRα, Liver X nuclear receptor alpha; COX, Cyclooxygenase; TAF1, TATA box binding protein-associated factor 1; ABCA1, ATP-binding cassette transporter ABCA1; ABCG1, ATP-binding cassette sub-family G member 1; GAPDH, Glycerinaldehyde-3-phosphat-dehydrogenase

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Introduction

Mesenchymal stromal cells (MSCs) are multipotential non-hematopoietic progenitor cells capable of differentiating into multiple lineages of the mesenchyme. In bone marrow, these niche stromal cells provide a sheltering microenvironment controlling the proliferation and differentiation of hematopoietic stem cells [1]. Well documented immunomodulatory properties of MSCs include the inhibition of T cell proliferation as well as modulation of other immune cells, e.g., B, natural killer, dendritic, and cytotoxic T cells, maintaining them in an immature and/or arrested state [1,2]. Consistent with the promising immunological properties in vitro, MSCs have been shown to display a considerable therapeutic potential in pre-clinical [3–7] and clinical [8–14] studies for the treatment of neuronal degeneration, osteogenesis imperfecta, graft versus host disease, support of hematopoietic engraftment, metabolic diseases, bone and cartilage diseases as well as renal and myocardial infarction.

So far, no serious side effects or adverse effects have been reported in any of the published clinical trials. In contrast to studies in man, long-term partial maldifferentiation of intraglomerular MSCs into adipocytes, accompanied by glomerular sclerosis, have been reported in a progressive rat model of glomerulonephritis [15]. The direct injection of large numbers of MSCs into the renal artery as used in this model can result in abrupt deterioration of renal blood flow, resulting in infarcts and loss of kidney function. Because those reported maldifferentiations might threaten the further use of MSCs in cellular therapies, efforts concentrate on preclinical studies which could assure an as high as possible safety in using in vitro expanded MSCs.

We have previously shown that human MSCs expanded in platelet lysate (PL)-supplemented medium exhibited decreased lipid accumulation under adipogenic induction as compared to cells cultured in the presence of fetal calf serum (FCS) [16]. Still, these MSCs met all current criteria defining a MSC population [17]. Thus, on the one hand the use of PL provided a preferred expansion method by excluding xenogenic proteins and transfer of potentially dangerous contaminations. On the other hand, the reduced adipogenic potential provided by this method might contribute to an increased safety in using MSCs for cellular regeneration in several applications.

In our previous experiments we performed microarray analyses and identified genes differentially regulated by FCS versus PL culture supplementation. Interestingly, we observed that lipocalin-type prostaglandin D2 synthase (L-PGDS), a putative regulator of adipogenesis, was several fold less expressed in MSCs expanded with PL compared to FCS [16]. The goals of the present study were to confirm the robustness of the anti-adipogenic effect of PL using MSCs from additional donors, and to gain mechanistic insight how PL modulates adipogenesis by studying adipogenic and lipogenic genes. In addition, we tested the functional role of L-PGDS in adipogenesis of MSCs.

Materials and methods

Materials

Plastic cell culture materials were obtained from Gibco (Karlsruhe, Germany), cell culture reagents and anti-GAPDH antibody from Novus Biologicals (Acris, Herford, Germany), L-PGDS protein and anti-L-PGDS antibody from Cayman Chemical (Ann Arbor, MI, USA), Reverse Transcriptase, OptiMEM I and the C18 fatty acid analog BODIPY® 500/510C1, C12 (BODIPY) from Invitrogen (Darmstadt, Germany), RNeasy Mini Kit and Quantitect predesigned L-PGDS primers from Qiagen (Hilden, Germany), TaqMan Assay-on-Demand primer sets from Applied Biosystems (Darmstadt, Germany), Microcon-membranes from Millipore (Schwalbach, Germany), siRNA from Ambion (Austin, TX, USA), DharmaFECT from Fisher Scientific (Schwerte, Germany), SYBR Premix Ex Taq from Takara Bio (Mobitec, Göttingen, Germany), protease inhibitor mix from Roche (Roche, Mannheim, Germany), chemiluminescence reagent RPN2132 from GE Healthcare (GE Healthcare, Munich, Germany) and Protran nitrocellulose membrane from Whatman (Whatman Schleicher-Schüll, Dassel Germany). AKT and pAKT antibodies were purchased from Cell Signaling Technology (New England Biolabs GmbH, Frankfurt, Germany).

Isolation and expansion of human MSCs

Bone marrow samples (BM) from healthy donors were collected after informed consent according to the hospital's guidelines approved by the Hamburg Ethics Committee and processed as described [16]. Briefly, MSCs were expanded in parallel in growth medium containing either 10% FCS or 5% PL. Cells were fed twice a week with fresh medium and incubated until reaching confluency of ≈90%. At this time point the cultures were designated as passage 0 (P0). Cells were detached with 0.05% Trypsin-EDTA and seeded at 500 cells/cm^2 as P1 or frozen in liquid nitrogen until further use. Human MSCs of passage 1 were characterized for expression of surface markers CD90, CD105, CD59, CD45 and CD34 and multipotential differentiation capabilities into adipogenic, osteogenic and chondrogenic lineages as described [18].

Adipogenic differentiation and quantification of lipid staining

Parallel P2-P3 cultures of FCS- and PL-supplemented MSCs of 3 individual donors were differentiated into adipocytes as described [18]. Briefly, confluent MSCs were repeatedly treated in differentiation cycles consisting of growth medium supplemented with 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, 100 μM indomethacin, and 10 μM insulin (differentiation medium) for 2–3 day, unless indicated otherwise, followed by 1 day with insulin in growth medium only (maintenance medium). In each experiment the last differentiation cycle was not completed and cells were harvested after incubation with differentiation medium. For lipid staining, cells were fixed with 4% paraformaldehyde for 1 h at room temperature (RT), washed 3 times with 60% isopropanol, air-dried and stained with 0.35% Sudan Red in isopropanol for 10 min. After 4 washes with aqua dest. the cells were air-dried again. Sudan Red was dissolved in 500 μl pure isopropanol for 10 min and the optical density (OD) determined at 500 nm. Pure isopropanol was used as control.