**Urokinase receptor mediates mobilization, migration, and differentiation of mesenchymal stem cells**


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**Aims**

Multipotent mesenchymal stem cells (MSCs) have regenerative properties and are recognized as putative players in the pathogenesis of cardiovascular diseases. The underlying molecular mechanisms remain, however, sparsely explored. Our study was designed to elucidate a probable role for the multifunctional urokinase (uPA)/urokinase receptor (uPAR) system in MSC regulation. Though uPAR has been implicated in a broad spectrum of pathophysiological processes, nothing is known about uPAR in MSCs.

**Methods and results**

uPAR was required to mobilize MSCs from the bone marrow (BM) of mice stimulated with granulocyte colony-stimulating factor (G-CSF) in vivo. An insignificant amount of MSCs was mobilized in uPAR \(^{-/-}\) C57BL/6j mice, whereas in wild-type animals G-CSF induced an eight-fold increase of mobilized MSCs. uPAR \(^{-/-}\) mice revealed up-regulated expression of G-CSF and stromal cell-derived factor 1 (CXCR4) receptors in BM. uPAR down-regulation leads to inhibition of human MSC migration, as shown in different migration assays. uPAR down- or up-regulation resulted in inhibition or stimulation of MSC differentiation into vascular smooth muscle cells (VSMCs) correspondingly, as monitored by changes in cell morphology and expression of specific marker proteins. Injection of fluorescently labelled MSCs in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice after femoral artery wire injury demonstrated impaired engraftment of uPAR-deficient MSCs at the place of injury.

**Conclusions**

These data suggest a multifaceted function of uPAR in MSC biology contributing to vascular repair. uPAR might guide and control the trafficking of MSCs to the vascular wall in response to injury or ischaemia and their differentiation towards functional VSMCs at the site of arterial injury.

**Keywords**

Urokinase receptor • Mesenchymal stem cells • Cell mobilization • Cell migration • Cell differentiation

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1. **Introduction**

Stem cells have been found in nearly every tissue where they are responsible for natural and pathological regeneration and repair processes. Mesenchymal stem cells (MSCs) resident to the bone marrow (BM) are of special interest. MSCs are mesodermal cells, which mainly reside in the BM and are mobilized to the blood circulation after injuries and chronic pathological vascular changes. They migrate and adhere to the affected tissue, where they proliferate and finally, when cued by the appropriate microenvironment, differentiate into tissue-specific cells. They give rise to muscle, brain, liver cartilage, bone, and fat. Recent studies report the ability of MSCs to differentiate additionally into cardiomyocytes and vascular smooth muscle cells (VSMCs), affecting by this way the outcome of cardiovascular diseases. These properties of MSCs make them an attractive tool for cell-mediated gene therapy in several disease processes including tissue repair after vascular injury. Despite the increasing body of investigations, numerous questions related to the mobilization of MSCs, their proposed pluripotency, and their role in vascular remodelling and repair in vivo are—in contrast to haematopoietic stem cells (HSCs)—still largely unanswered.

Among multiple factors regulating pathophysiological processes upon vascular remodelling after injury, an important role for the multifunctional urokinase (uPA)/urokinase receptor (uPAR) system has been
documented.\textsuperscript{5} uPAR was originally identified as a cellular receptor for its natural ligand uPA, which mediates the conversion of plasminogen to plasmin and thereby regulation of cell surface proteolysis in space and time.\textsuperscript{6} Meanwhile, it is known that independently of proteolytic processes uPAR regulates migration, adhesion, proliferation, and differentiation of various cell types in tissue regeneration through activation of an intracellular signalling network.\textsuperscript{7} uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein that can be shed from the cell surface. Recent reports provide evidence that soluble uPAR (suPAR) affects HSC migration and that the serum level of suPAR is up-regulated during granulocyte-stimulating factor (G-CSF)-induced HSC mobilization.\textsuperscript{8,9} Cell surface membrane uPAR was implied as an important regulator of HSC adhesion and migration in the BM.\textsuperscript{10}

The involvement of the uPA/uPAR system in regulating MSC properties and functions remains, however, unexplored. Therefore, our study was designed to investigate the role of the uPA/uPAR system in MSC regulation. We provide evidence that uPAR is required for MSC mobilization from the BM, wound healing, and differentiation of MSCs into VSMC-like cells.

2. Methods

2.1 Cell culture

Human BM MSCs were obtained from Lonza (Lonza Walkersville, Inc.). These cells have been well characterized by the manufacturer for their surface markers and differentiation potential. Flow cytometry analysis showed that these cells were positive for CD105, CD166, CD29, and CD44, but negative for CD34, CD14, and CD45. MSCs were cultured and expanded in MSC growth medium (MSCGM) as recommended by the supplier and were used between passages 6 and 7. To induce MSC differentiation, MSCGM was replaced when MSCs reached 80\% confluence with smooth muscle (SM) inducing medium consisting of MSC basal medium (MSCBM, Lonza Walkersville, Inc.), supplemented with 2\% fetal bovine serum, 5 ng/mL transforming growth factor-\beta (TGF-\beta) (R&D Systems), 4 mM l-glutamine (Lonza Walkersville, Inc.), and 0.01\% penicillin/streptomycin (Biochrom AG, Germany). The medium was changed every 2 days and induction of cell differentiation was carried out for 7 days.

2.2 Plasmid construction and lentiviral infection

Lentiviral siRNA (small interfering RNA) vectors were designed and cloned as described previously.\textsuperscript{11} Lentiviral vectors were produced by transient transfection of human embryonic kidney-293T cells and used for MSC infection as described.\textsuperscript{12} For uPAR overexpression, lentiviral vector pWPTS-GFP (kindly provided by Didier Trono, Department of Genetics and Microbiology, Faculty of Medicine, University of Geneva, Switzerland) was modified by ligating synthetic oligonucleotide duplex in BamHI and NotI restriction sites. Resultant vector was designated as pWPTS-Ad. Final pWPTS-uPAR lentiviral vector was generated by ligating Sall and NotI-digested pWPTS-Ad together with uPAR cDNA.

2.3 Quantitative real-time polymerase chain reaction analysis

Human total RNA and mouse total RNA were isolated from MSCs and mouse BM using QIAGEN QiaSpin miniprep kit (QIAGEN, Hilden, Germany) and DNAse kit (QIAGEN), respectively, according to the manufacturer’s suggested protocol. Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed on a LightCycler\textsuperscript{\textregistered} 480 Real-Time PCR System using LightCycler\textsuperscript{\textregistered} 480 RNA Master Hydrolysis probes (Roche Diagnostics GmbH, Mannheim, Germany).

2.4 Flow cytometry

Cells were detached from culture plates with 0.04\% Trypsin/0.03\% EDTA (PromoCell GmbH, Heidelberg, Germany), fixed with 0.2\% paraformaldehyde in PBS for 10 min, washed, and permobilized in 1\:1 concentration of acetonemethanol for 20 min on ice, washed, and stained with corresponding primary antibodies [smooth muscle alpha-actin (\alpha-SMA), Sigma; 1:500, calponin, Sigma; 1:500, SM22a, AbCam; 1:5000] and with the secondary fluorescein-labelled antibody Alexa Fluor\textsuperscript{\textregistered} 488 (Molecular probes). Flow cytometry was performed using FACS CANTO system (BD Biosciences, USA) using acquisition software FACS DIVA (BD Biosciences, USA). Data were analysed using Summit software (Dako, Denmark).

2.5 Cell migration assay

MSC migration was studied in a wound-healing assay, modified Boyden chamber, and fluorescence-based quantitative scratch wound healing assay using MSCs with down-regulated uPAR and control cells.

2.6 Animal studies

uPAR-deficient mice (uPAR\textsuperscript{-/-}) and corresponding C57BL/6 wild-type (WT) mice were bred under pathogen conditions in the animal facility of Phenos GmbH, Hannover, Germany. NOD.CB17- Prkdc scid\textsuperscript{j} mice, homozygous for the severe combined immune deficiency spontaneous mutation Prkdc and characterized by an absence of functional T cells and B cells, lymphopenia, and hypogammaglobulinaemia, commonly referred to as scid mice, were purchased from Charles River, Sulzfeld, Germany. All these mice, male, were used at 8–12 weeks of age. For all surgical procedures, the mice were anaesthetized by inhalation anaesthesia with 2\% isoflurane. Experiments were performed according to the guidelines for care and use of laboratory animals approved by the institutional ethical animal care committee of the Medical School of Hannover. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.7 Statistical analysis

All the experiments were performed in triplicate. Data from all experiments are presented as mean ± standard deviation. Statistical analysis was performed using Student’s t-test.

Detailed description of Methods is presented in Supplementary data online.

3. Results

3.1 MSC mobilization from the BM is impaired in uPAR-deficient mice

A few previous reports documented a capability of cleaved and membrane forms of uPAR to regulate HSC trafficking from and to BM.\textsuperscript{8–10} No data are, however, available regarding involvement of uPAR in mobilization of MSCs. In this study, we thus aimed at investigating the possibility of uPAR requirement for MSC mobilization. We used the model of cell mobilization from the BM in response to G-CSF\textsuperscript{13,14} and examined whether loss of uPAR may affect the mobilizing capability of MSCs. In steady-state conditions, the number of circulating MSCs in the peripheral blood was comparable in WT and uPAR\textsuperscript{-/-} mice. Administration of G-CSF caused an eight-fold increase of mobilized MSCs in WT but not in uPAR\textsuperscript{-/-} mice (Figure 1A), thus indicating that uPAR is a necessary participant for the MSC mobilization process in response to G-CSF. To exclude the fact that changes in the MSC amount were related to uPAR-mediated cell adhesion, additional experiments were performed

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using a cell adhesion assay. No significant difference in cell adhesion was observed for control and uPAR-deficient MSCs (see Supplementary Figure S1).

To get an idea about the mechanisms behind impaired MSC mobilization in uPAR-deficient mice, we analysed expression levels of G-CSF, G-CSFR, and CXCR4 in the uPAR−/− and WT mice. Interestingly, we found that uPAR deficiency resulted in up-regulation of G-CSF in the plasma of uPAR−/− mice compared with WT animals (Figure 1B, left panel). Expression of G-CSFR (G-CSFR; Figure 1B, right panel) and CXCR4 receptor (C), in bone marrow of WT and uPAR−/− mice before and after stimulation with G-CSF were assessed by TaqMan analysis. Increase in G-CSFR and CXCR4 in uPAR−/− mice, though being of statistically low relevance, was observed already in animals not stimulated with G-CSF. G-CSF application, as expected, significantly reduced expression of both receptors in WT mice. Remarkably, this reduction was far less pronounced in uPAR−/− mice that may explain why MSC mobilization from the BM is impaired in uPAR−/− animals.

3.2 uPAR is required for MSC migration

In the next experimental setting, we aimed at elucidating whether uPAR might be required for migratory properties of MSCs. Migration experiments were performed in a wounding assay, modified Boyden chamber, and fluorescence-based quantitative scratch wound healing assay using MSCs with down-regulated uPAR and control cells. uPAR silencing resulted in impaired MSC migration when compared
that in response to TGF-β stimulation MSCs acquire a VSMC-like phenotype with a classical hills and valleys pattern of the cell culture (Figure 3A, lower panel). TGF-β stimulation induced up-regulation of SM markers in MSCs. In particular, we observed a strong increase in expression of SM α-actin, calponin, and SM22α proteins, as shown by flow cytometry (Figure 3B) and western blot analysis (Figure 3D). Up-regulated expression of these markers at the mRNA level was confirmed by TaqMan experiments (Figure 3D). In immunocytochemistry, increased expression of contractile proteins was paralleled by up-regulated immunoreactivity of the respective antigens at the cellular level (Figure 4).

### 3.4 uPAR mediates MSC differentiation into VSMCs

We asked whether uPAR would mediate MSC differentiation into VSMC lineage. We observed that uPAR expression in MSCs stimulated for differentiation was up-regulated (Figure 5A). To examine whether uPAR might control the up-regulation of SM markers in MSCs, uPAR was down-regulated in these cells by means of a lentivirus-based interfering RNA. As shown in Figure 5B, uPAR silencing resulted in decreased expression of contractile proteins when compared with MSCs infected with control viruses. To further investigate uPAR requirement for MSC–VSMC differentiation, uPAR expression was additionally up-regulated by means of a lentiviral vector system. As expected, uPAR overexpression induced increases in expression of SM α-actin, calponin, and SM22α (Figure 5C).

### 3.5 uPAR is required for MSC engraftment at the place of vascular injury

To examine whether our findings might be relative for repair processes after vascular injury, we relied on a corresponding animal model. Transplantation of fluorescently labelled human MSCs in immunodeficient NOD/SCID mice after femoral artery wire injury was performed. Human MSCs with down-regulated uPAR and control cells were double stained with enhanced green fluorescent protein (eGFP) and the lipophilic cell membrane dye (CM-DiI) and injected retro-orbitally 4 h after the surgery. Fluorescently labelled control MSCs carrying competent uPAR were detected 24 h after transplantation at the site with control cells, in both the wound closure assay and the Boyden chamber (Figure 2A and B). This effect was observed already in the absence of uPA and significantly more pronounced in MSCs stimulated with uPA. To examine what signalling pathways might underlie the observed changes in MSC migration capacities, we performed inhibitory studies. MSC treatment with AG 1295, a specific inhibitor for tyrosine kinase activity of platelet-derived growth factor receptor (PDGFR), resulted in inhibition of uPA-induced but not basal cell migration. Similar results were obtained after cell treatment with wortmannin which specifically targets the PI3-K pathway (Figure 2C). In contrast, no changes in MSC migration were observed in response to inhibition of CXCR4 and rho-associated protein kinase (ROCK)-related signalling (data not shown).

### 3.3 MSCs differentiate into VSMC-like cells

MSCs are multipotent progenitor cells with the capacity to differentiate into different tissue cell types such as chondrocytes, osteocytes, and adipocytes. Recent reports showed evidence of an ability of MSCs to differentiate into VSMCs-like cells,15–20 though this differentiation process is far from being characterized and the underlying molecular mechanisms remain undefined.

To address the question of a probable involvement of uPAR in MSC–VSMC differentiation, experimental conditions for this process have been first established. Several conditions have been reported to drive the differentiation of MSCs towards the VSMC lineage depending on the MSC origin and other parameters varying in experimental protocols.21 We tested MSC differentiation upon stimulation with TGF-β, ascorbic acid, and a combination of both in an MSCBM medium (Lonza Walkersville, Inc.) as well as in Mesenpro medium (Invitrogen). Additionally, MSCs were stimulated for differentiation using a VSMC medium (Lonza) and VSMCs-conditioned medium. MSC stimulation with TGF-β in the MSCBM medium was the most effective approach available to us and was finally selected and used in all experiments.

Classically, MSCs are identified by a CD44+/CD105+ phenotype. In response to TGF-β stimulation, this characteristic phenotype was impaired. Thus, expression of both markers became increasingly negative (Figure 3A, upper and middle panels). Cell morphology confirmed
MSCs undergo differentiation into VSMC-like cells. (A) Differentiation of MSCs to VSMC-like cells was induced by TGF-β stimulation. Representative flow cytometric analysis shows down-regulation of MSC markers CD105 and CD44 upon stimulation (upper and middle panels, respectively). Morphological changes were observed between cells without and with TGF-β after 7 days (lower left and right panels, respectively). $n=3$. (B) Representative flow cytometric analysis shows up-regulation of VSMC markers α-SMA, calponin, and SM22α upon stimulation. $n=3$. (C) Expression of VSMC markers in MSCs upon stimulation was assessed by western blotting with GAPDH as loading control. Graph represents the quantification of bands normalized with GAPDH in un-stimulated and stimulated cells. **$P<0.001$; $n=3$. (D) Relative RNA expression of α-SMA and calponin in un-stimulated and stimulated cells assessed by TaqMan analysis. **$P<0.001$; $n=3$. 

**Figure 3** MSCs undergo differentiation into VSMC-like cells. (A) Differentiation of MSCs to VSMC-like cells was induced by TGF-β stimulation. Representative flow cytometric analysis shows down-regulation of MSC markers CD105 and CD44 upon stimulation (upper and middle panels, respectively). Morphological changes were observed between cells without and with TGF-β after 7 days (lower left and right panels, respectively). $n=3$. (B) Representative flow cytometric analysis shows up-regulation of VSMC markers α-SMA, calponin, and SM22α upon stimulation. $n=3$. (C) Expression of VSMC markers in MSCs upon stimulation was assessed by western blotting with GAPDH as loading control. Graph represents the quantification of bands normalized with GAPDH in un-stimulated and stimulated cells. **$P<0.001$; $n=3$. (D) Relative RNA expression of α-SMA and calponin in un-stimulated and stimulated cells assessed by TaqMan analysis. **$P<0.001$; $n=3$. 

* uPAR in MSCs function
of vascular injury. In experiments with uPAR-deficient MSCs, injected cells failed to engraft the injury vessel wall (Figure 6).

4. Discussion

Currently, an increasing body of evidence points to MSCs as promising candidates for regenerative and immunomodulatory cell therapy.32

MSCs reside in BM and can also be mobilized in response to stimulation with blood cytokines, such as G-CSF.23,24 Transplantation studies show that BM-derived MSCs enter the circulation and engraft other tissues, including artery wall, at sites of injury.3 BM-derived MSCs also have vascular differentiation potential. In culture, they differentiate into VSMC- and endothelial cell-like cells.25–27 In vivo, BM-derived cells that were seeded on a synthetic vascular graft produced SM and endothelial layers.28 In animal studies and clinical trials for cardiac regeneration therapy after post-myocardial infarction, intramyocardial injection or intravenous administration of autologous MSCs increased vasculogenesis and improved cardiac function.29,30 Other interesting studies reported the potential of MSCs to contribute to atherosclerosis or post-stenting restenosis with beneficial effects on attenuation of neointima growth.31

Evidence shows that MSCs have a higher potential to differentiate into cells with a muscular phenotype, compared with HSCs.31 For HSCs, however, lineage acquisition is regulated by well-defined colony-stimulating factors. Little is known about the corresponding regulatory mechanisms for MSCs. In our studies, we analysed conditions for cultivation and differentiation of MSCs and achieved the most reliable MSC differentiation into VSMCs by cell stimulation with TGF-β, experimental conditions reported also by others.21 We observed under these conditions induction of the SMC-selective genes, such as SMα-actin, SM22α, and calponin, at both protein and mRNA levels, as well as in immunocytochemical studies. Of interest, the results of experiments aimed at uPAR targeting in MSCs by siRNA and uPAR overexpression provide evidence that activation of SMC marker genes was uPAR dependent. These results are the first to provide direct evidence that uPAR plays an important role in the development of VSMCs from the multipotential MSCs.

The uPA/uPAR system is an active participant in the development of cardiovascular diseases and vascular remodelling.32–36 The underlying molecular and cellular mechanisms are multifaceted including regulation of cell surface-associated proteolysis, orchestration of signalling pathways underlying functional behaviour of vascular cells, and immunomodulatory capacities.7,37,38

Figure 4 Immunocytochemical analysis of VSMC markers in differentiated MSCs. MSCs were grown in MSCBM supplemented with 2% fetal bovine serum on glass cover slips without (A, D, and G) or with (B, E, and H) the addition of TGF-β. Immunofluorescence staining was performed for α-SMA (red), calponin (green) (D and E) and SM22α (green) (G and H) on day 7. Nuclei were stained with diamidino-2-phenylindole (DAPI) (blue). Anti-mouse Alexa Fluor® 594 (C), Alexa Fluor® 488 (F), and anti-rabbit Alexa Fluor® 488 (I) labelled secondary antibodies are shown for secondary antibody controls. n = 3.
Figure 5 uPAR mediates differentiation of MSCs to VSMC-like cells. (A) Expression of uPAR in un-stimulated and stimulated MSCs was assessed by western blotting with GAPDH as loading control. Graph represents the quantification of bands normalized with GAPDH in un-stimulated and stimulated cells. ***P < 0.0002; n = 3. Representative western blots (B and C, middle and right panels) show down- and up-regulation of VSMC markers α-SMA, calponin, and SM22α upon uPAR silencing and overexpression, respectively, (B and C, left panels) in MSCs stimulated with TGF-β. Graph represents the quantification of bands normalized with GAPDH in stimulated cells. **P < 0.005; n = 3.

Figure 6 MSC engraftment at the site of arterial injury is impaired in uPAR-deficient cells. Control (upper panels) and uPARsi (lower panels) MSCs were pre-labelled with eGFP (green, A and E) and CM-Dil (red, B and F) and injected retro-orbitally into NOD/SCID mice 4 h after femoral artery wire injury. Twenty-four hours after injury, the arteries were perfusion fixed and paraffin embedded. Nuclei were stained with DAPI (blue, C and G); merged images are shown in D and H. Arrows indicate double positive cells attached to the internal elastic lamina in injured artery sections. n = 5–6 mice/group.
uPAR requirement for fibroblast-to-myoﬁbroblast differentiation and epithelial–mesenchymal transition has been reported. We observed earlier that uPAR is an active regulator of VSMC phenotypic modulations. Interestingly, in VSMCs uPAR served for VSMC transition from their physiological contractile to the pathophysiologically de-differentiated phenotype, that was initiated by uPAR-directed down-regulation of SMC-speciﬁc genes. In contrast, in the present study, we observed an opposite effect of uPAR, namely, its requirement for induction of SMC-speciﬁc genes upon MSC–VSMC differentiation. This different functional outcome of uPAR on cell differentiation might be a result of a well-known cell type speciﬁcity of uPAR-mediated pathways. The identiﬁcation of the molecular machinery utilized by uPAR to induce MSC differentiation is a topic for further interesting research. The next issue to be addressed is the functionality of MSCs differentiated into VSMC-like cells, which still remains questionable. Recent study showed that despite speciﬁc SMC protein expression and modiﬁcation of electrophysiological properties similar to that of VSMCs, MSCs cultured in a differentiation medium failed to display contractile properties.

Migration of MSCs to areas of pathology is a critical step in tissue regeneration. New evidence suggests that the artery wall is a recipient of MSCs. We found that uPAR was required for MSC migration as well as for mobilization from the BM, uPAR down-regulation resulted in decrease of MSC migration and retardation of wound closure pointing to involvement of uPAR in mechanisms affecting MSC motility and wound healing. Our data are in agreement with other recent studies demonstrating that the uPA/uPAR system mediates MSC motility, in particular, tropism of MSCs and neural cells to solid tumours. The mechanisms underlying uPAR necessity for cell migration are, as already mentioned, cell type speciﬁc. In VSMCs, uPAR signals a migratory behaviour through Tyk2, PI3-K, and the Rho family of proteins. The results of our inhibitory studies indicate that PDFGR tyrosine kinase and PI3-K are the probable candidates for propagating migration signal in MSCs downstream of uPAR.

Our in vivo experiments using injection of ﬂuorescently labelled MSCs with down-regulated uPAR after vascular injury in NOD/SCID mice, provide evidence that the uPAR-directed migration potential of MSCs was required for cell engraftment at the place of injury. Though, as mentioned above, MSC recruitment to injured tissues has been reported, our data shed further light on the underlying molecular mechanisms pointing to a role of uPAR in this process. However, the elucidation of the molecular cascade, by which uPAR regulates MSC migration and engraftment needs further studies.

Tissue repair and regeneration after injury involves the selective mobilization of stem cells from BM and recruitment of circulating or resident stem cell populations. Our in vivo experiments using uPAR-deﬁcient mice revealed a surprisingly strong attenuation of MSC mobilization from the BM into circulation in response to G-CSF in these animals. Though MSC mobilization by blood cytokines including G-CSF has been reported, the regulatory and controlling pathways of this process remain elusive. Our ﬁndings indicate that uPAR may provide a tool for the study of MSC mobilization and homing. What is the molecular mechanism of impaired MSC mobilization in uPAR−/− mice? At least two possibilities may be suggested—(i) uPAR loss leads to inhibition of migratory cell properties and (ii) uPAR is required to mediate induction of MSC mobilization of itself. Though we observed uPAR requirement for MSC migration properties, inhibition of MSC mobilization in uPAR−/− mice is likely due to the fact that uPAR generates signals that regulate stem cell trafficking. Thus, we observed strong up-regulation of G-CSF, G-CSFR and CXCR4 in uPAR−/− mice which clearly points to the deregulation of the mobilization process. A high level of endogenous G-CSF in uPAR−/− mice before stimulation may lead to desensitization and explain, together with up-regulated G-CSFR and CXCR4 expression, why additional stimulation with G-CSF did not initiate MSC mobilization.

Together, our data clearly demonstrate that uPAR is causally involved at multiple steps in the MSC biology. Our ﬁndings are among the ﬁrst reports conﬁrming that targeting the uPA/uPAR system may be promising in the MSC-based therapy.

### Supplementary material

Supplementary material is available at Cardiovascular Research online.

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### Conﬂict of interest

None declared.

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