Impaired recruitment of HHT-1 mononuclear cells to the ischaemic heart is due to an altered CXCR4/CD26 balance

Simone Post1,2†, Anke M. Smits1†, Alexandra J. van den Broek1, Joost P.G. Sluijter1, Imo E. Hoefer1, Ben J. Janssen3, Repke J. Snijder4, Johannes J. Mager4, Gerard Pasterkamp1,2, Christine L. Mummery2,5, Pieter A. Doevendans1,2, and Marie-José Goumans1*

1Department of Cardiology, Division Heart & Lung, UMCU, Utrecht, the Netherlands; 2Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands; 3Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, the Netherlands; 4Department of Pulmonary Disease, St Antonius Hospital, Nieuwegein, the Netherlands; and 5Department of Anatomy and Embryology, LUMC, Leiden, the Netherlands

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Aims

Mononuclear cells (MNCs) from patients with hereditary haemorrhagic telangiectasia type 1 (HHT1), a genetic disorder caused by mutations in endoglin, show a reduced ability to home to infarcted mouse myocardium. Stromal cell-derived factor-1α (SDF-1α) and the chemokine receptor CXCR4 are crucial for homing and negatively influenced by CD26. The aim of this study was to gain insight into the impaired homing of HHT1-MNCs.

Methods and results

CXCR4 and CD26 expression on MNCs was determined by flow cytometry. Transwell migration to SDF-1α was used to analyse in vitro migration. Experimentally induced myocardial infarction in mice, followed by tail vein injection of MNCs, was applied to study homing in vivo. HHT1-MNCs expressed elevated levels of CXCR4, but this was counterbalanced by high levels of CD26, resulting in decreased migration towards an SDF-1α gradient in vitro. Migration was enhanced by inhibiting CD26 with Diprotin-A. While MNCs from healthy controls responded to transforming growth factor-beta stimulation by increasing CXCR4 and lowering CD26 expression levels, HHT1-MNCs did not react as efficiently; in particular, CD26 expression remained high. Inhibiting CD26 on MNCs increased the homing of human cells into the infarcted mouse heart. Interestingly, the defect in homing of HHT1-MNCs was restored by pre-incubating the HHT1-MNCs with Diprotin-A before injection into the tail vein.

Conclusion

We show that a decreased homing of HHT1-MNCs is caused by an impaired ability of the cells to respond to SDF-1α. Our results suggest that modulating CD26 levels using inhibitors like Diprotin-A can restore homing in cases where increased expression of CD26 contributes to the underlying pathological mechanism.

Keywords

Mononuclear cells • Chemokines • Genetics • Myocardial infarction • Migration

1. Introduction

Hereditary haemorrhagic telangiectasia (HHT, also known as Rendu-Osler-Weber disease) is an autosomal dominant vascular disorder with an estimated prevalence of approximately 1 in 10 000. Several different types of HHT have been described, but characteristically they are all associated with mutations in components of the transforming growth factor-beta (TGFβ) signalling pathway. The underlying cause of HHT type 1 (HHT1) are mutations in endoglin (CD105), an accessory TGFβ type III
CD26 impairs homing of HHT-1 mononuclear cells

receptor. Endoglin is primarily expressed on proliferating endothelial cells in vitro and angiogenic blood vessels in vivo and also on other cell types, such as circulating blood mononuclear cells (MNCs) although to a lesser extent. Clinically, HHT1 is characterized by telangectasias and epistaxis. With age, the incidence and severity of bleedings increases. Because endoglin is crucial for the formation of new vessels, the higher number of haemorrhages in the endoglin haplo-insufficient HHT1 patients may result from a reduced ability to restore the injured vasculature. Vascular repair is mediated by activation of endothelial cells lining the vessel wall to replace the damaged cells, but it has become evident that circulating MNCs also have the ability to restore damaged vessels. Vessel repair and the influx of MNCs, including monocytes and lymphocytes, are important in the restoration of the injured heart, for example after a myocardial infarction (MI). MNCs promote healing of the damaged heart via stimulation of myofibroblast proliferation, deposition of collagen and the stimulation of angiogenesis. Recently, we demonstrated that the recruitment of human MNCs to the infarcted murine heart and subsequent vessel formation is severely impaired when using HHT1-derived MNCs compared with healthy MNCs.

Homing and trafficking of cells is regulated to a large extent by the chemokine stromal-cell-derived factor-1 (SDF-1) and its receptor CXCR4. SDF-1 co-internalizes with CXCR4. CD26 therefore interferes with the extracellular portion of CXCR4. Additionally, CD26 can cleave the amino-terminal dipeptide from SDF-1 or CXCL12) and its receptor CXCR4. SDF-1 is upregulated in ischaemic myocardial tissue. MNCs promote healing of the damaged heart via stimulation of myofibroblast proliferation, deposition of collagen and the stimulation of angiogenesis. Recently, we demonstrated that the recruitment of human MNCs to the infarcted murine heart and subsequent vessel formation is severely impaired when using HHT1-derived MNCs compared with healthy MNCs.

Since all mutations in the endoglin gene reported to date result in reduced functional cell surface protein levels and deregulation of TGFβ signalling, we hypothesized that this mutation alters the balance between CXCR4 and CD26 on HHT1-MNCs. This would lead to an impaired ability of HHT1-MNCs to respond to elevated SDF-1α levels in vitro and in vivo.

2. Methods

2.1 Patients and blood samples

Venous blood samples from age- and gender-matched healthy human volunteers and HHT1 patients were collected in potassium/EDTA tubes (Vacuette, Greiner Bio-One, the Netherlands). Peripheral blood MNCs were isolated by density gradient centrifugation using Ficoll Paque Plus (Amersham Biosciences, Sweden), according to the manufacturer’s protocol. Isolated MNCs were washed twice with PBS supplemented with 2 mM EDTA, and counted on a hemocytometer. All procedures were approved by the medical ethics committee of the St Antonius Hospital Nieuwegein, the Netherlands. The investigation conforms to the principles outlined in the Declaration of Helsinki.

2.2 Flow cytometry

Flow cytometric analysis was performed using 100 μL of whole blood or 3 × 10^5 MNCs in PBS. Whole blood was stained with anti-CD14-PE (DakoCytomation, Denmark), anti-Endoglin-Fluorescein (R&D Systems, USA), anti-CD34-FITC (BD Pharmingen, USA), and anti-VEGFR2-PE (R&D Systems). MNCs were stained with anti-CD14-ECRD (Immunotech, Coulter, France), anti-CD26-FITC (Serotec, UK), and anti-CXCXR4-PE (BD Pharmingen). Isotype-matched fluorochrome-conjugated antibodies were used as controls. Red blood cells were lysed before measuring fluorescence on a flow cytometer (Cytomics FC500, Beckman Coulter, the Netherlands). Analysis was performed using CXP software (Beckman Coulter). The number of positive cells is expressed as absolute cell number per millilitre of whole blood, or as percentage of positive cells within a cell fraction. The mean-fluorescent intensity (MFI) is presented for cell populations of interest.

2.3 MNC migration

Migration of freshly isolated MNCs was assessed in a transwell system using polycarbonate filters with 5 μm pores (Corning, the Netherlands). Prior to migration, MNCs were incubated for 1 h in RPMI 1640 Glutamax medium, supplemented with 10% FBS at 37°C. If applicable, MNCs were stained with Calcein AM (Invitrogen, Karlsruhe, Germany). MNCs were pre-treated at room temperature for 15 min with 5 mM Diprotin-A (Sigma-Aldrich, St Louis, USA) to inhibit CD26 or for 30 min at room temperature with 5 μg/mL AMD3100 (Sigma, Saint Louis, USA) to block CXCR4. One hundred thousand MNCs were applied to the upper well, and in the lower well medium, without or with 200 ng/mL SDF-1α (PeproTech, Rocky Hill, NJ, USA), was added. The cells were allowed to migrate for 3 h at 37°C.

After migration, cells were collected and 75,000 PeakFlowTM carmine flow cytometry reference beads (6 μm, Invitrogen, Eugene, OR, USA) were added. The number of MNCs per 10,000 beads was assessed by flow cytometry. The migration percentage was calculated from the number of cells migrated to SDF-1α compared with the number of cells migrated in the absence of SDF-1α.

2.4 MNC stimulation

Freshly isolated MNCs were stimulated for 24 h at 37°C with 2 ng/mL TGFb1 (PeproTech, Rocky Hill) in RPMI medium supplemented with 1% FBS, and pre-treated for 15 min at room temperature with 5 mM Diprotin-A (Sigma-Aldrich) to inhibit CD26. Stimulated MNCs were stained with anti-CD14-ECRD, anti-CD26-Fluorescein, and anti-CXCXR4-PE. Expression levels of CXCR4 and CD26 were determined by measuring MFI. Expression changes were calculated by divid-
2.5 Induction of myocardial infarction in mice

All experiments were carried out in accordance with 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 and with prior approval by the Animal Ethical Experimentation Committee, Utrecht University. In 28 BALB/cOla-Hsd mice (Harlan, the Netherlands), aged 10–12 weeks, a MI was induced as described previously.22 Briefly, mice were intubated and ventilated with 2% isoflurane/98% oxygen. A left thoracotomy was performed and the left anterior descending (LAD) coronary artery was visualized. The LAD was permanently occluded by placing a 7-0 prolene suture.

2.6 Intravenous injection of human MNCs

One day after induction of MI, BALB/c mice received 5*10^6 human MNCs (HHT1 or control, n = 7 per group) via tail vein injection. Mice were immunosuppressed by subcutaneous injection of tacrolimus (5 mg/kgd) for 4 days.

2.7 Tissue collection

Five days after MI, surviving mice were euthanized (CTL−: n = 5, CTL+Dip: n = 6, HHT−: n = 7, HHT+Dip: n = 5). The hearts were flushed with 5 mL of PBS via the right ventricle and dissected. The tissue was processed for cryosectioning in OCT compound (Sakura, the Netherlands).

2.8 Immunohistochemistry

Frozen longitudinal 7 μm thick sections of the whole ventricle were stained using a mouse anti-human nuclei antibody (Chemicon, Temecula, CA, USA) for the identification of human cells. Briefly, sections were fixed in acetone, air dried and rehydrated in PBS. Endogenous peroxidase activity was blocked, followed by incubation with avidin and biotin, respectively. The tissue was permeabilized in 0.2% Triton X-100 in PBS and blocked with 3% BSA in PBS for 30 min. During this incubation, the anti-human nuclei antibody was biotinylated 1:50 using the Dako-ARK for mouse primary antibodies (Dako, the Netherlands) according to the manufacturer’s protocol. Sections were incubated with α-human nuclei, PBS, or mouse IgG1 (Dako) as an isotype control overnight at 4°C. After washing, slides were incubated with ABC peroxidase complex (DAKOcytomation, Dako) at room temperature for 30 min. Peroxidase activity was detected using AEC substrate. After counterstaining with haematoxylin, sections were mounted in gelatin/glycerin.

2.9 Semi-quantitative polymerase chain reaction for human Alu sequences

Genomic DNA was isolated by pooling 10–50 cryosections (10 μm) followed by incubation in 500 μL lysis buffer (0.1 M Tris–HCl pH 8.5, 5 mM EDTA, 0.2% SDS, 0.2 M NaCl). Genomic DNA was extracted using phenol-chloroform, and precipitated with ethanol. The polymerase chain reaction (PCR) primers were described previously and are specific for human Alu sequences, resulting in a product of 224 basepairs.23 PCR was carried out using 50 ng of total DNA under the following conditions: 95°C for 10 min, followed by the appropriate number of cycles of 95°C for 30 s, 58°C for 45 s, 72°C for 45 s, and 72°C for 10 min. Amplification of DNA isolated from mice receiving Diproton-A treated or untreated MNCs was optimized at 23 and 27 cycles, respectively. The samples were run on a 10% polyacrylamide gel and quantified using Quantity One software (Biorad, the Netherlands).

2.10 Statistics

Statistical significance was evaluated using the Mann-Whitney U test for comparison between two independent samples, Wilcoxon signed rank test for two related samples, Spearman’s rho for correlation calculations and Fisher’s exact test for comparison of two categorized variables, using SPSS v11.0 for Windows. Results are expressed as mean± standard error of the mean (SEM). A value of P < 0.05 was considered statistically significant.

3. Results

3.1 Circulating cell populations

To establish whether the attenuated response of HHT1 to ischemic injury is due to fewer circulating cells capable of participating in restoration of vascular damage, we compared peripheral blood of HHT1 patients to age- and gender-matched healthy controls (see Supplementary material online, Tables S1 and S2 for patient characteristics). Monocytes (defined as CD14+) are circulating cells that are able to home to damaged tissue and activated monocytes are known to express endoglin.24 We found no difference in the number of circulating CD14+ cells (Figure 1A and B), and observed low levels of endoglin expression on circulating CD14+ monocytes that decreased in HHT1 patients (Figure 1C and D).

Other cells that can contribute to vascular repair are those within the CD34 expressing cell fraction. Endothelial progenitor cells (EPCs) are part of this CD34+ population, and one way to identify EPCs may be as CD34+–VEGFR2+ cells. In contrast to our expectation, HHT1 patients show a significantly higher number of CD34+ cells (Figure 1E and F), but the number of CD34+–VEGFR2+ cells is comparable between HHT1 patients and controls (Figure 1G and H).

3.2 Distribution of CXCR4 and CD26

The CXCR4/VEGF-1α axis is particularly important for cell homing, chemotaxis, engraftment, and retention in ischemic tissues. Analysing the distribution of CXCR4 on MNCs (Figure 2A) showed that, although the number of cells in the MNC population expressing CXCR4 was not different between groups (Figure 2B), the mean expression level of CXCR4 on the cell surface (represented by MFI) was significantly lower on HHT1-MNCs (Figure 2D, E and F). Additionally, within the MNC subpopulations, lymphocytes and monocytes demonstrated similar patterns in the expression of CXCR4 and CD26 (Supplementary material online, Figure S1A and B).

3.3 HHT1-MNCs show impaired migratory response in vitro

CD26 can regulate SDF-1α/CXCR4 mediated chemotaxis; therefore, we performed a migration assay using SDF-1α as a chemottractant. HHT1-MNCs exhibited decreased migration towards SDF-1α compared with controls (Figure 3A). Interestingly, while pre-treatment with the CXCR4 inhibitor AMD3100 completely
blocked the migration of both control and HHT1-MNCs, pre-treatment with the CD26 inhibitor Diprotin-A only significantly improved the migration of the HHT1-MNCs compared with untreated MNCs (Figure 3B and C).

Analysing the CXCR4 and CD26 expression on migrated cells revealed that the number of CXCR4⁺ MNCs positively correlated with the migration capacity for both control as well as HHT1-MNCs (Figure 3D – F). Interestingly, the mean CD26 expression on HHT1-MNCs was negatively correlated with their migratory capacity (Figure 3G), while the migration of control MNC did not correlate with CD26 levels (Figure 3E).

3.4 MNC response to TGFβ stimulation
TGFβ can play a role in the homing and migration of cells to damaged tissue. After MI, TGFβ levels are increased in the ischaemic area. Since in HHT1 patients, TGFβ signalling is disturbed and their homing capacity is abrogated, we investigated the effect TGFβ stimulation has on the CXCR4 and CD26 expression levels on MNCs. Although flow cytometric analysis of TGFβ stimulated MNCs revealed a pronounced increase in total percentage of CXCR4 expressing cells as well as the mean CXCR4 expression on both HHT1 and control MNCs (Figure 4A and B), the relative induction of receptor levels was less on HHT1-MNCs than on control MNCs (3.4-fold for HHT1 vs. 5.1-fold for control MNCs, Figure 4C). After TGFβ stimulation, the total number of CD26 expressing cells, as well as the mean expression level, was reduced in both control and HHT1-MNCs (Figure 4D and E).

Importantly, the percentage of CD26 positive cells was higher within HHT1-MNCs than within control MNCs, even after TGFβ stimulation (Figure 4D and E). Since CD14⁺ monocytes might have angiogenic activity, we analysed the effect TGFβ has on these cells. CD14⁺-monocytes showed similar TGFβ-induced CXCR4 expression (Supplementary material online, Figure S1D) and reduced CD26 expression (Supplementary material online, Figure S1E). These experiments show that endoglin haploinsufficiency results in decreased TGFβ responsiveness. Although HHT1-MNCs are able to respond to TGFβ stimulation by increasing CXCR4 and down regulating CD26 expression, this occurred to a lesser extent, and the net result is a decreased migratory capacity to SDF-1α compared with controls.

3.5 in vivo MNC homing to myocardial infarction
To evaluate the importance of the increased expression of CD26 on MNCs in HHT1 patients for homing and migration to damaged tissue, we used the mouse MI model. Mice underwent a permanent ligation of the LAD. Since SDF-1α levels reach a maximum 24 h post-MI, 1 day after MI the mice received an intravenous injection of human MNCs, which were pre-treated or not with the CD26 inhibitor Diprotin-A. Mice were sacrificed 5 days after MI to assess MNC homing. Immunohistochemical staining using an anti-human nuclei antibody revealed human cells scattered throughout the injured myocardium 5 days after infarction (Figure 5A – C).
number of human cells found in the myocardium was quantified by semi-quantitative PCR for human specific Alu repeats (Figure 5D). Five days post-MI, a significantly lower amount of human DNA was found in the myocardium of mice injected with HHT1-MNCs when compared with those receiving untreated control MNCs (Figure 5E, 0.15 ± 0.05 and 0.29 ± 0.06 arbitrary units, respectively, see Supplementary material online, Table S3). Strikingly, when human MNCs were pre-treated with Diprotin-A, there was no longer a difference in the homing and engraftment into the infarcted heart between HHT1 patients and controls (Figure 5F, 0.64 ± 0.18 vs. 0.55 ± 0.22 for HHT1 and controls, respectively, see Supplementary material online, Table S3). These results underline the importance of CD26 expression for the homing and migratory capacity of MNCs.

4. Discussion

One of the hallmarks of HHT1 is an increasing frequency of haemorrhages with age, which can be the result of impaired vascular repair. Restoration of vasculature is initiated by a local rapid increase in chemotactic chemokines followed by homing of cells to the site of injury. Previously, we have shown that the HHT1-MNC population had a reduced ability to accumulate and induce vessel formation in the infarcted region of the heart. Here we demonstrate that this observed defect in homing cannot be explained by changes in the numbers of angiogenic cells known to participate in tissue repair within the heterogeneous MNC population. Analysis of the MNC composition using flow cytometry did not show a significant difference in the number of circulating CD14⁺-monocytes or CD34⁺-VEGFR2⁺ cells. HHT1 patients did have a significantly higher number of CD34⁺ cells, which may be the result of diffuse vascular damage in HHT-1 patients, resulting in increased CD34⁺ progenitor cell mobilization. Similarly, increases in CD34⁺ cell numbers were found in patients after MI. Progenitor cell numbers do not seem to be the cause of the HHT1-MNC dysfunction. Therefore, an alternative explanation for the reduced cell numbers in the ischaemic heart may be an impaired chemotactic response of the circulating HHT1-MNCs rather than reduced circulating MNC numbers.

Myocardial wound healing is a tightly controlled process and can be divided into distinct phases. One recognizable phase is the infiltration of MNCs into the infarct area, inducing blood vessels growth, myofibroblast proliferation, and extracellular matrix production. An important mechanism for cell recruitment to ischaemic areas is the formation of an SDF-1α gradient, resulting in the mobilization of circulating cells expressing its cognate receptor CXCR4. Binding of SDF-1α to CXCR4 was shown to be essential for mobilization and migration of different cell types, e.g. hematopoietic stem cell (HSC), monocytes/mesothelial cells, and tumour metastasis. Since SDF-1α expression is increased as early as 1 h after induction of hypoxia in the myocardium, it is believed to play a role in the initiation of tissue repair. More importantly, modulating the SDF-1α/CXCR4 axis, either positively by...
delivering a protease-resistant SDF-1α or negatively, by inhibiting SDF-1α binding to its receptor using the CXCR4 antagonist AMD3100, was shown to influence the recruitment and engraftment of cells in infarcted myocardium as well as myocardial repair. Furthermore, the expression of SDF-1α serves as a retention signal and is crucial for the engraftment and maintenance of pro-angiogenic CXCR4 expressing cells within the tissue. The lower number of HHT1-MNCs within the infarcted myocardium can be explained by reduced numbers of CXCR4+ cells or reduced CXCR4 expression per cell. However, we found no difference between HHT1 patients and controls. In HHT1 patients, the mean expression of CXCR4 on the cell-surface was even higher than on control MNCs. Therefore, the reduced number of HHT1-MNCs found in the injured heart cannot be explained by a reduction in CXCR4 levels.

Although CXCR4 expression levels are known to be important for migration, our data imply that this alone does not predict the migratory behaviour of cells to SDF-1α. SDF-1α-mediated chemotaxis is also regulated by the cell surface peptidase CD26, which cleaves the amino-terminus of SDF-1α. This N-terminal cleavage will block its binding to CXCR4 and limit the effectiveness of SDF-1α as a chemotactant in the inflammatory environment of infarcted myocardium. CD26 has been reported to be expressed by several cell types within the MNC fraction, including CD14+ and CD34+ cells. Analysis of CD26 indicated that the HHT1-MNCs have higher expression levels of CD26 per cell. These increased CD26 levels suggest that although the high CXCR4 levels on HHT1-MNCs would imply increased recruitment of cells to ischaemic tissue, their homing capacity is negatively influenced by the high CD26 expression levels. Using a transwell migration assay, we indeed found a decreased chemotactic response of the HHT1-MNCs towards SDF-1α when compared with healthy controls. Furthermore, the migration of HHT1 cells to SDF-1α was significantly improved when the activity of CD26

**Figure 3** Migration of MNCs *in vitro*. The potential of MNCs to migrate to SDF-1α was determined in a transwell migration system. (A) HHT1-MNCs have a decreased capacity to migrate to SDF-1α, compared with healthy controls. (B and C) The effect of pre-treatment of MNCs with AMD3100 or Diprotin-A on migration. Depicted is the relative migration capacity towards SDF-1α compared with untreated cells. MNC migration positively correlates with the number of CXCR4+ cells shown by a positive Spearman’s rho (D and F). However, only HHT1-MNC migration negatively correlates with CD26 expression levels (E and G). CTL, n = 17; HHT1, n = 17; AMD3100 CTL, n = 5; HHT1, n = 4; correlations CTL, n = 15; HHT1, n = 17. *P < 0.05; **P < 0.03; ***P < 0.005. Bars show mean ± SEM.
was blocked by Diprotin-A; a tri-peptide which was previously shown to increase homing of embryonic stem cells and HSCs to SDF-1. Additionally, we show that the effect of CD26 on cell migration is negatively correlated with its expression level. The inability of Diprotin-A to influence the migration of control MNCs is probably due to low CD26 levels on these cells that did not correlate with their migratory behaviour capacity. Our results clearly demonstrate that the balance of CD26 levels in relation to CXCR4 expression levels is of great importance to predict the chemotactic response of MNCs. The increased number of high CD26 expressing cells in the HHT1 population, causing a misbalance between CD26 and CXCR4, may have a major impact on their total homing and retention capacity.

As a consequence of the endoglin mutations underlying HHT1, TGFβ signalling is impaired. TGFβ has previously been reported to influence the SDF-1α/CXCR4 axis, by increasing the expression of CXCR4 and decreasing the CD26 levels on tumour and mesothelial cells. We investigated the response of the MNCs to TGFβ stimulation. TGFβ stimulation increased the surface expression of CXCR4 on control MNCs, whereas HHT1-MNCs show impaired induction of CXCR4 expression and impaired reduction in CD26 levels. These observations suggest that the imbalance in the CXCR4/CD26 axis we observe in these cells could be caused by an impaired response of HHT1-MNCs to TGFβ stimulation. Therefore, when HHT1-MNCs are exposed to stress signals after MI, such as TGFβ, their capacity to shift the balance between CXCR4 and CD26 is disturbed, thereby rendering the cells less capable for migration and homing. The relatively high CXCR4 expression observed on unstimulated HHT1-MNCs might be a compensatory mechanism to deal with the high CD26 expression on these cells.

Since in vitro analysis of the HHT1-MNCs indicated CD26 imbalance as an explanation for impaired homing, we interfered with its expression. Previous studies have shown that pre-treatment of HSCs with Diprotin-A greatly enhanced their homing capacity towards the bone marrow. Therefore, we investigated whether altering the CXCR4/CD26 balance, by inhibiting CD26 with Diprotin-A, also increased their homing in vivo. When untreated MNCs were injected 1 day after MI, we found significantly fewer HHT1-MNCs in the infarcted area 5 days after MI, compared with healthy controls. This confirmed the impaired homing capacity we reported previously, as well as, the in vitro data presented in this study. Strikingly, pre-treatment with Diprotin-A completely normalized the homing of HHT1-MNCs to the infarcted heart, indicating the significance of CD26 for homing not only for cells towards the bone marrow but also for MNCs to ischaemic tissue.

In conclusion, the SDF-1α/CXCR4 axis and its negative regulator CD26 are crucial for the homing of MNCs to the ischaemic myocardium. In HHT1 patients, the balance of CXCR4 and CD26 on HHT1-MNCs is skewed. Restoring the balance in this axis, via CD26 inhibition, resulted in ‘normal’ homing capacities in vitro and in vivo, providing an explanation for the dysfunctional homing of HHT1-MNCs. Interestingly, CD26 inhibition may not
only be of importance for HHT, but also for other diseases that aim at regeneration of ischaemic areas by improving homing and engraftment of circulating cells into the injured organ.

**Supplementary material**

Supplementary Material is available at Cardiovascular Research Online.

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