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CD26/DPP-4 inhibition recruits regenerative stem cells via stromal cell-derived factor-1 and beneficially influences ischaemia-reperfusion injury in mouse lung transplantation[†]

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Abstract

OBJECTIVES: The CD26 antigen is a transmembrane glycoprotein that is constitutively expressed on activated lymphocytes and in pulmonary parenchyma. This molecule is also identified as dipeptidyl peptidase-4 (DPP-4) that cleaves a host of biologically active peptides. Here, we aimed to identify an important substrate of CD26/DPP-4—stromal cell-derived factor-1 (SDF-1/CXCL12)—as a key modulator for stem-cell homing together with its receptor CXCR4 in response to ischaemic injury of the lung.

METHODS: Orthotopic single lung transplantation (Tx) was performed between syngeneic C57BL/6 mice. Inhibition of CD26/DPP-4 activity in recipients was achieved using vildagliptin (10 mg/kg, every 12 h) subcutaneously, and 6 h ischaemia time was applied prior to implantation. Forty-eight hours after Tx, lung histology, SDF-1 levels (enzyme-linked immunosorbent assay) in lung, spleen and plasma, and expression of the SDF-1 receptor CXCR4 in blood and lung were assessed. Homing of regenerative progenitor cells to the transplanted lung was evaluated using fluorescent-activated cell sorting.

RESULTS: Compared with untreated lung transplanted mice, systemic DPP-4 inhibition of Tx recipients resulted in an increase in protein concentration of SDF-1 in plasma, spleen and lung. Concordantly, the frequency of cells bearing the SDF-1 receptor CXCR4 rose significantly in the circulation and also in the lungs of DPP-4-inhibited recipients. We found co-expression of CXCR4/CD34 in the grafts of animals treated with vildagliptin, and the stem-cell markers Flt-3 and c-kit were present on a significantly increased number of cells. The morphology of grafts from DPP-4 inhibitor-treated recipients revealed less alveolar oedema when compared with untreated recipients.

CONCLUSIONS: Targeting the SDF-1–CXCR4 axis through CD26/DPP-4 inhibition increased the intragraft number of progenitor cells contributing to the recovery from ischaemia-reperfusion lung injury. Stabilization of endogenous SDF-1 is achievable and may be a promising strategy to intensify sequestration of regenerative stem cells and thus emerges as a novel therapeutic concept.

Keywords: Ischaemia-reperfusion injury • Orthotopic mouse lung transplantation • CD26/DPP-4 • SDF-1 • CXCR4 • Progenitor cells

INTRODUCTION

CD26 is a type II transmembrane glycoprotein that exerts potent co-stimulatory effects on signalling lymphocytic activation [1]. This molecule has an extra-membranous catalytic domain, dipeptidyl peptidase-4 (DPP-4), that possesses exopeptidase activity. DPP-4 is constitutively expressed on many haematopoietic cells including activated T- and B-lymphocytes, capillary endothelial cells [2], epithelial cells and stem cells [3], but is also found in a catalytically

active soluble form in plasma [4]. DPP-4 cleaves N-terminal dipeptides from peptides where usually proline or alanine resides at the penultimate position. A wide range of chemokines, growth factors, neuropeptides and other key biological peptides are subject to cleavage by DPP-4 [5]. In the wake of investigating the *in vivo* catalytic activity of DPP-4, we recently identified pulmonary vasoactive intestinal peptide (VIP) as crucial for ameliorating ischaemia reperfusion (I/R) in a rat transplantation (Tx) model [6]. This concept could be reproduced in the mouse model of lung Tx where the inhibition of DPP-4 intragraft enzymatic activity led to an enhancement of VIP levels and a preservation and profound regeneration of the pulmonary function [7].

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Among many other peptides that are cleaved by DPP-4, the chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) constitutes the most important protein for the recruitment and homing of bone marrow-derived regenerative stem cells throughout the mammalian system [8]. Along with its receptor CXCR4, it forms an axis that is highly conserved in humans and mice [9]. The modulation of the SDF-1 and CXCR4 axis was demonstrated to improve stem-cell engraftment following injury [10–12]. This mechanism of stem-cell homing is not restricted to a specific organ. Concentrations of SDF-1 are increased in bleomycin lung injury [13] and, upon hypoxia, SDF-1 reacts with heightened CXCR4 expression resulting in an enhanced chemoattractive response [14]. Furthermore, experimental evidence suggested that pharmacological inhibition of DPP-4 improved heart function [15] and increased survival in a heterotopic heart Tx model [16].

We hypothesize here that inhibition of DPP-4 retards the degradation of SDF-1 and thereby (i) stabilizes systemic and intra-graft levels of SDF-1, (ii) recruits regenerative progenitor cells to the site of injury through an enhanced SDF-1–CXCR4 axis and (iii) improves the recovery of the transplanted organ. In order to address these questions, we orthotopically transplanted single lungs between syngeneic BL/6 mice, determined protein contents of SDF-1 in circulation and Tx lungs, analysed the circulating and intra-graft CXCR4 expression and regenerative progenitor cells, and finally evaluated the outcome of the Tx grafts after 48 h. The groups of analysis comprised: wild-type animals that were neither transplanted nor inhibitor-treated (WT); CD26 knockout animals that were also neither transplanted nor inhibitor-treated (CD26/DPP-4 KO); single lung-transplanted animals (Tx); and single lung-transplanted animals that received the DPP-4-inhibitor vildagliptin subcutaneously (Tx-inhibited).

MATERIALS AND METHODS

Mice

Specific pathogen-free inbred male mice, strain C57BL/6 (H2b) (Harlan, Horst, The Netherlands), weighing 25–32 g, were used. CD26/DPP-4 KO mice (on a C57BL/6 background) were obtained from Taconic (Taconic, Bomholt, Denmark) with approval from Dr D. Marguet (Centre d'Immunologie de Marseille Luminy-INSERM, Marseille Luminy, France) [17]. Animals received adequate care in strict accordance with the Principles of Laboratory Animal Care (National Institutes of Health Publication No. 85-23, promulgated in 1985, most recently revised in 1996), and the study was approved by the local veterinary ethical committee under the study number 177/2010.

Experimental setting

Orthotopic, single-lung Tx between C57BL/6 mice was performed as described before [18]. Treated animals ($n=3-6$) received vildagliptin (custom synthesized by GLSynthesis, Inc., Worcester, MA, USA), at a dose of 10 mg/kg (diluted in 0.9% NaCl solution to achieve a final injection volume of 400 μ l) subcutaneously immediately before Tx, and every 12 h thereafter (Tx-inhibited), whereas controls ($n=3-6$) did not receive any treatment upon transplantation (Tx). The donor graft was exposed to 6 h cold ischaemia time before transplantation. All

grafts were analysed 48 h after Tx, animals receiving neither DPP-4 inhibitor treatment nor undergoing lung transplantation are indicated as wild-type animals (WT) and CD26/DPP-4 knock-out animals (CD26/DPP-4 KO).

Lung recovery

Before sacrifice, animals were intubated, anaesthetized and a laparosternotomy was performed as described [18]. Ventilation was maintained applying a tidal volume of 1 ml with a positive end-expiratory pressure of 2 mbar remaining unchanged until removal of the heart and lungs. The lungs were flushed with 10 ml of 0.9% normal saline solution at a pressure of 10 cm H₂O via the pulmonary artery, and organs were subsequently removed.

Magnetic resonance imaging

Magnetic resonance (MR) imaging of the mouse lung was conducted on a 4.7 T small animal scanner (Bruker BioSpec, Switzerland) using a transmit-receive radiofrequency coil with a three-dimensional ultrashort echo time sequence.

Histology, H&E

Tx lungs and Tx-inhibited lungs were fixated in 4% phosphate-buffered formalin, cut and embedded in paraffin. Sections of 4 μ m thickness were cut and stained for haematoxylin & eosin (H & E).

DPP-4 activity assay

DPP-4 enzymatic activity was assayed in mouse plasma and lung homogenates (homogenization protocol as described earlier [2]) using glycyl-prolyl-4-methoxy- β -naphthylamide (Gly-Pro-4-Me- β -NA) as fluorogenic substrate according to Scharpé *et al.* [19]. In a 96-well plate, 5- μ l samples were mixed with 0.5 mM Gly-Pro-4-Me- β -NA in 50 mM Tris buffer, pH 8.3, in a final volume of 110 μ l. DPP-4 activity was determined kinematically for 10 min at 37°C by measuring the velocities of 4-Me- β -NA release ($\lambda_{ex}=340$ nm, $\lambda_{em}=430$ nm) from the substrate using an Infinite™ 200 (Tecan Group Ltd, Switzerland). Fluorescence intensity was related to a 4-Me- β -NA standard curve. The reversibility of the DPP-4 inhibitor, vildagliptin, in the samples and the dilution of these samples in the assay result in an underestimation of the DPP-4 inhibition. Therefore, a calibration curve was created with known concentrations of the inhibitor in mouse plasma to estimate the percentage of the *in vivo* inhibition (all reagents are from Sigma-Aldrich, Germany).

Enzyme-linked immunosorbent assay for SDF-1

Plasma, lung and spleen were collected at autopsy and stored at –80°C. Spleen and lungs were homogenized in ice-cold lysis buffer [1% Triton X-100 in Dulbecco's phosphate-buffered saline, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA) and protease inhibitor cocktail (P-2714; Sigma-Aldrich (1:10)]. Fifty milligrams of the tissue were homogenized in 50 μ l of lysis

buffer using a micro tissue grinder. The material was then centrifuged for 10 min at 14 000 rpm and 4°C. The protein concentration in the collected supernatant was estimated using the DC protein assay (Bio-Rad Laboratories, Reinach, Switzerland) with bovine serum albumin as a standard. To assess the concentration of CXCL12/SDF-1 α , we used the Quantikine sandwich enzyme-linked immunosorbent assay from R&D system (cat MCX120; detection limit of 156 pg/ml). We tested 50 μ l of diluted tissue lysate containing 0.5 mg of proteins. The remaining steps were performed according to the manufacturer's protocol. All results were expressed in pg/mg protein for lung and spleen analysis.

Flow cytometry of peripheral blood and single cells from lung transplants

Single-cell suspensions from transplanted lungs were prepared as follows: lungs were meshed and collected in 5 ml of Roswell Park Memorial Institute (RPMI) with 10% foetal calf serum and 50 μ g/ml gentamicin, after which they were incubated in 0.5 ml of collagenase (147 units/mg) (PAN Biotech, Aidenbach, Germany) (2 mg/ml collagenase II in RPMI) for 30 min at 37°C. The collagenase reaction was stopped by adding 1 ml of 1 M EDTA and cells were then filtered through a 70- μ m cell strainer. Cells and full blood samples were stained with following antibodies: CD45-PB, CD26-FITC, CD34-FITC, CD184 (CXCR4)-PE, CD117 (c-kit)-PE and Flt-3-PE (all Abs from BD Pharmingen, Allschwil, Switzerland) and subjected to flow cytometry using BD SORP flow cytometer, LSRII Fortessa. Between 30 000 and 200 000 events were analysed for each sample.

Statistics

Data analysis was performed using SPSS for Windows 15.0 (SPSS, Inc., Chicago, IL, USA). For group comparison of more than two groups, the Kruskal-Wallis test was performed, and to compare the differences between two groups, the Mann-Whitney *U*-test was performed. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

DPP-4 activity in plasma and lung homogenates

In order to prove that the subcutaneous application of the DPP-4 inhibitor vildagliptin had an effect not only in the peripheral circulation, but also in the lungs, we determined the activity of DPP-4 in plasma and lung homogenates (Fig. 1). In the WT animal, plasma DPP-4 activity (6.1 U/l), being the physiological activity, was the highest. The activity diminished after transplantation (2.5 U/l) and was even lower in a Tx-inhibited animal (1.3 U/l). The DPP-4 activity in plasma derived from a CD26/DPP-4 KO mouse was almost undetectable (0.3 U/l) (Fig. 1a). The percentage of *in vivo* DPP-4 activity in the Tx-inhibited animals compared with the Tx animals was estimated for both plasma and lung and is ~20 and 26%, respectively (Fig. 1b). This estimation is a way to compensate for the sample dilution and thus underestimation of the actual *in vivo* inhibition by the reversible DPP-4 inhibitor.

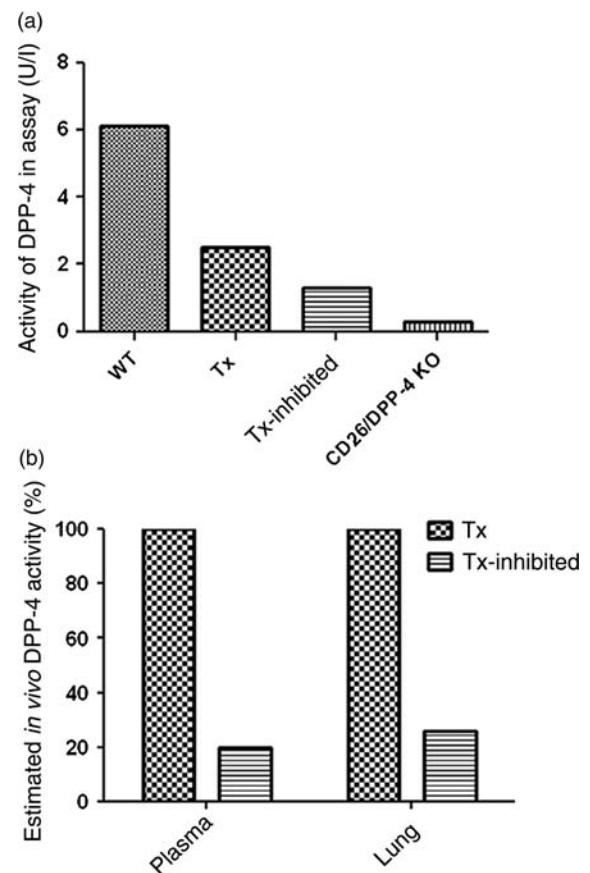


Figure 1: (a) Bar graphs indicating the plasma DPP-4 activity of a wild-type (WT), transplanted (Tx), Tx-inhibited and CD26/DPP-4 KO animal ($n=1$ for each condition). The activity was measured 12 h after the last subcutaneous application of 10 mg/kg vildagliptin. (b) Bar graphs indicating the estimated percentage *in vivo* DPP-4 activity in plasma and lung homogenates of a Tx and Tx-inhibited animal ($n=1$ for each condition). Plasma samples and lungs were collected 48 h after Tx.

CD26 receptor expression in peripheral blood

CD26 receptor expression on CD45⁺ haematopoietic cells showed an equal distribution between Tx (12.9%) and Tx-inhibited mice (11.9%) (Fig. 2).

SDF-1 protein levels in the lungs

WT lungs revealed low levels of SDF-1 protein (229 ± 36.8 pg/mg). In Tx animals, we found an increase in the SDF-1 concentration (480.1 ± 96.9 pg/mg), but grafts from Tx-inhibited animals showed significantly higher levels (715 ± 34.4 pg/mg; $P < 0.05$). Finally, lungs from CD26/DPP-4 KO mice had the highest concentration of SDF-1 (808.1 ± 37.8 pg/mg) (Fig. 3).

CXCR4 is enhanced in the peripheral blood of DPP-4 inhibitor-treated recipients

We observed a more than 9-fold increase in CXCR4⁺/CD45⁺ haematopoietic cells in peripheral blood of Tx-inhibited ($49.8 \pm 3.2\%$) compared with Tx animals ($5.6 \pm 1.1\%$) (Fig. 4). This difference was significant between both groups ($P < 0.05$).

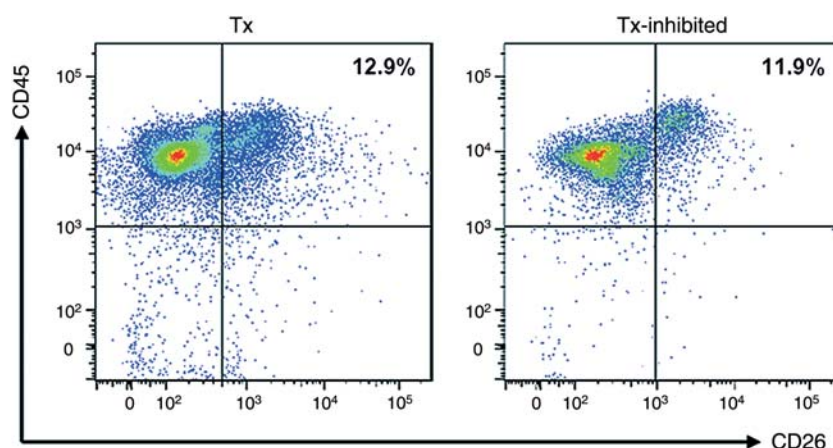


Figure 2: FACS analysis showing the percentages of the presence of the CD26-receptor on CD45⁺ cells in peripheral blood of transplanted (Tx) and Tx-inhibited animals taken 48 h after Tx ($n = 1$ for each condition).

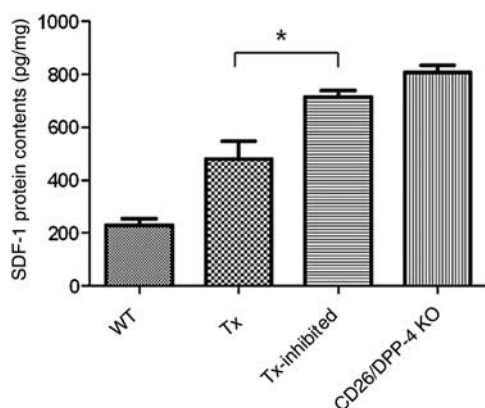


Figure 3: The SDF-1 protein levels in lung homogenates for different experimental conditions: wild-type (WT), transplanted (Tx), Tx-inhibited and CD26/DPP-4 KO. Data represent mean \pm SD ($n = 3$); $*P < 0.05$.

CXCR4 and CD34 are co-expressed in transplanted lungs of DPP-4 inhibitor-treated recipients

Not only in peripheral blood, but also in the transplanted lung, CXCR4 was expressed on CD45⁺ haematopoietic cells in significantly higher frequencies in Tx-inhibited animals ($14.7 \pm 3.5\%$) compared with Tx animals ($7.9 \pm 1.8\%$; $P < 0.05$). We were then interested in the presence of the marker of endothelial progenitor cells, CD34. When looking for its co-expression with CXCR4, we found a distinct cell population on CD45⁺ haematopoietic cells that displayed both markers at the cell surface. They homed into the transplanted lungs of Tx-inhibited mice ($16.6 \pm 2.2\%$) (Fig. 5).

Regenerative progenitor cells are increased in transplanted lungs of DPP-4 inhibitor-treated recipients

When staining the regenerative progenitor marker Flt-3 on CD45⁺ haematopoietic cells, they were found to be expressed in significantly higher frequencies in Tx-inhibited lungs ($12.8 \pm 1.8\%$) than in Tx lungs ($1.6 \pm 0.2\%$; $P < 0.05$) (Fig. 6). When additionally looking for the co-expression of Flt-3 and CD34, we

found a distinct cell population among the CD45⁺ haematopoietic cells that displayed both markers at the cell surface and that homed into Tx-inhibited lungs ($14.4 \pm 1.7\%$) (Fig. 6). Finally, the stem-cell marker c-kit (CD117) was found to be expressed significantly higher in Tx-inhibited lungs ($7.3 \pm 1.8\%$) compared with Tx lungs ($1.3 \pm 0.2\%$; $P < 0.05$) (Fig. 7).

Transplanted lungs of DPP-4 inhibitor-treated recipients display less ischaemic injury

The macroscopic appearance of syngrafted Tx lungs (Fig. 8a) vs. Tx lung from animals that were inhibited (Fig. 8b) appeared more inflamed and oedematous. MR images showed a lower transparency in Tx lungs (Fig. 8c) when compared with Tx lung from animals that were inhibited (Fig. 8d). When comparing the histological morphology of the transplanted lungs between the DPP-4 inhibitor-treated and untreated group, grafts from the untreated group showed a certain degree of congestion and considerable oedema of alveolar cells, whereas the grafts from the treated group showed a near-normal alveolar wall and cellular ultrastructure (Fig. 8e and f).

DISCUSSION

We provide evidence that pharmacological inhibition of the DPP-4 activity leads to (i) decreased systemic activity of DPP-4, (ii) stabilization of SDF-1 in the transplants, (iii) upregulation of its receptor CXCR4 in the transplants and (iv) homing of regenerative progenitor cells to the transplant with (v) better recovery from I/R injury.

As a proof of concept, we measured the activity of DPP-4 in plasma as well as lung homogenates. Inhibition of the DPP-4 activity by vildagliptin could be detected in both compartments. The plasma DPP-4 activity was higher in WT mice compared with Tx mice. The reason for the decreased activity upon syngeneic transplantation remains largely speculative. A similar decrease in plasma DPP-4 activity has been reported earlier in syngeneic heart transplantation, whereas in recipients of allogeneic grafts, plasma DPP-4 activity was increased [20]. Apart from the measurement of activity, fluorescent-activated cell sorting

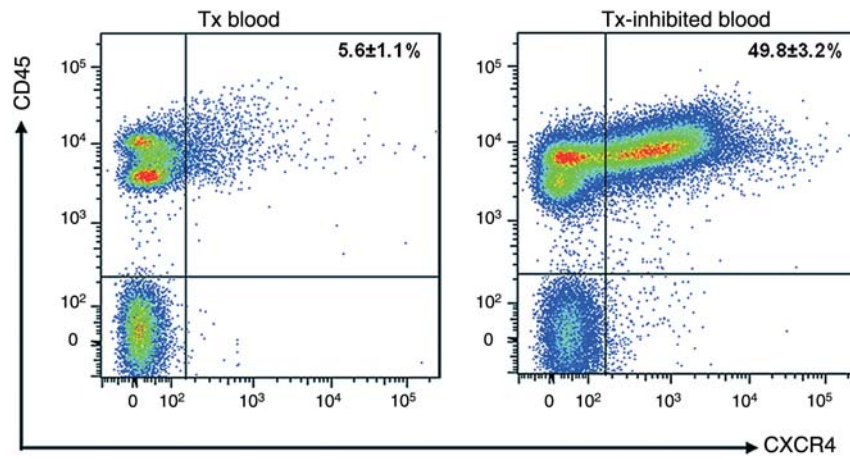


Figure 4: Representative FACS analysis showing the mean percentages of CD45⁺CXCR4⁺ cells in peripheral blood of transplanted (Tx) and Tx-inhibited animals taken 48 h after Tx, the latter showing a significantly higher cell frequency (**P* < 0.05); data represent mean ± SEM (*n* = 4).

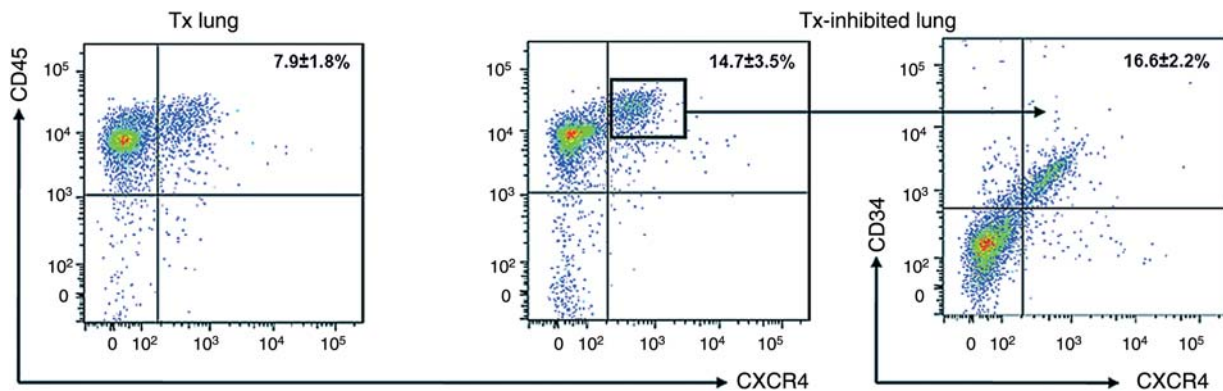


Figure 5: Representative FACS analysis showing the mean percentages of CD45⁺CXCR4⁺ cells in lung homogenates of transplanted (Tx) and Tx-inhibited animals, the latter showing a significantly higher cell frequency (**P* < 0.05); data represent mean ± SEM (*n* = 4). Gating of CD45⁺CXCR4⁺ cells for CD34 revealed a distinct population of CD34⁺CXCR4⁺ double positive cells in lung homogenates of Tx-inhibited animals.

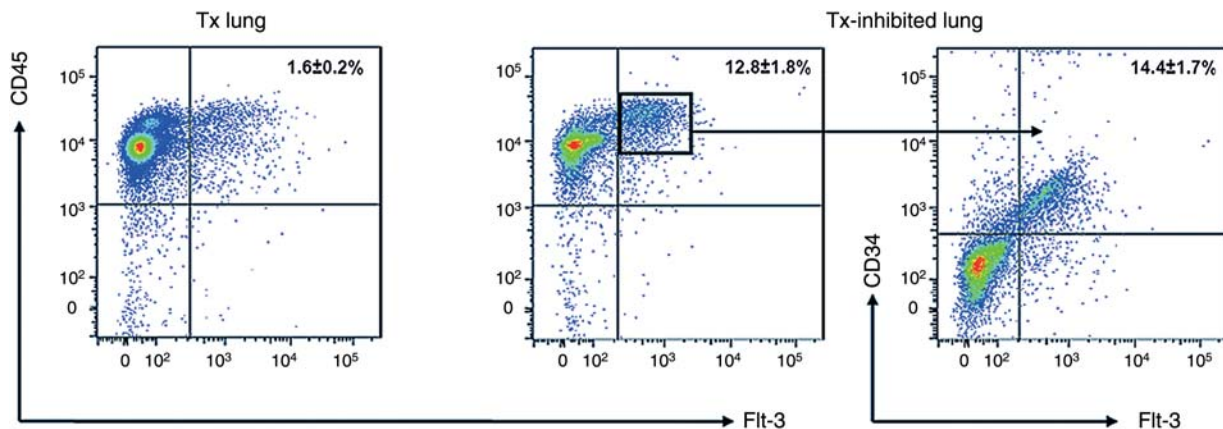


Figure 6: Representative FACS analysis showing the mean percentages of CD45⁺Flt-3⁺ cells in lung homogenates of transplanted (Tx) and Tx-inhibited animals, the latter showing a significantly higher cell frequency (**P* < 0.05); data represent mean ± SEM (*n* = 4). Gating of CD45⁺Flt-3⁺ cells for CD34 revealed a distinct population of CD34⁺Flt-3⁺ double positive cells in lung homogenates of Tx-inhibited animals.

(FACS) analysis of the CD26 protein expression on haematopoietic cells revealed an equal distribution in both, the Tx and Tx-inhibited mice. This finding suggests that vildagliptin does not affect the CD26 receptor expression on cells. The T-cell-mediated immune response, which is also involved in the

defence mechanism after I/R injury, is therefore believed to be essentially unaltered [1].

Under steady-state conditions, SDF-1 is not only constitutively expressed in spleen, but also present in small concentrations in the lung. However, levels are enhanced upon ischaemia and

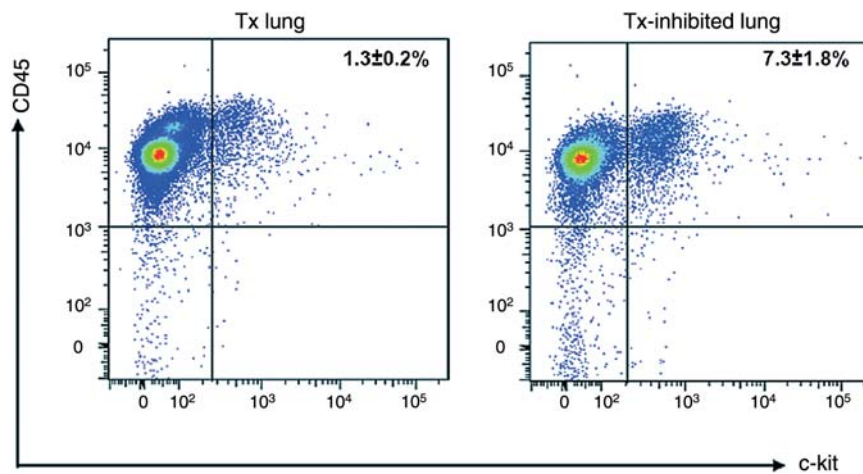


Figure 7: Representative FACS analysis showing the mean numbers of CD45⁺c-kit⁺ cells in lung homogenates of transplanted (Tx) and Tx-inhibited animals, the latter showing a significantly higher cell frequency (**P* < 0.05); data represent mean ± SEM (*n* = 4).

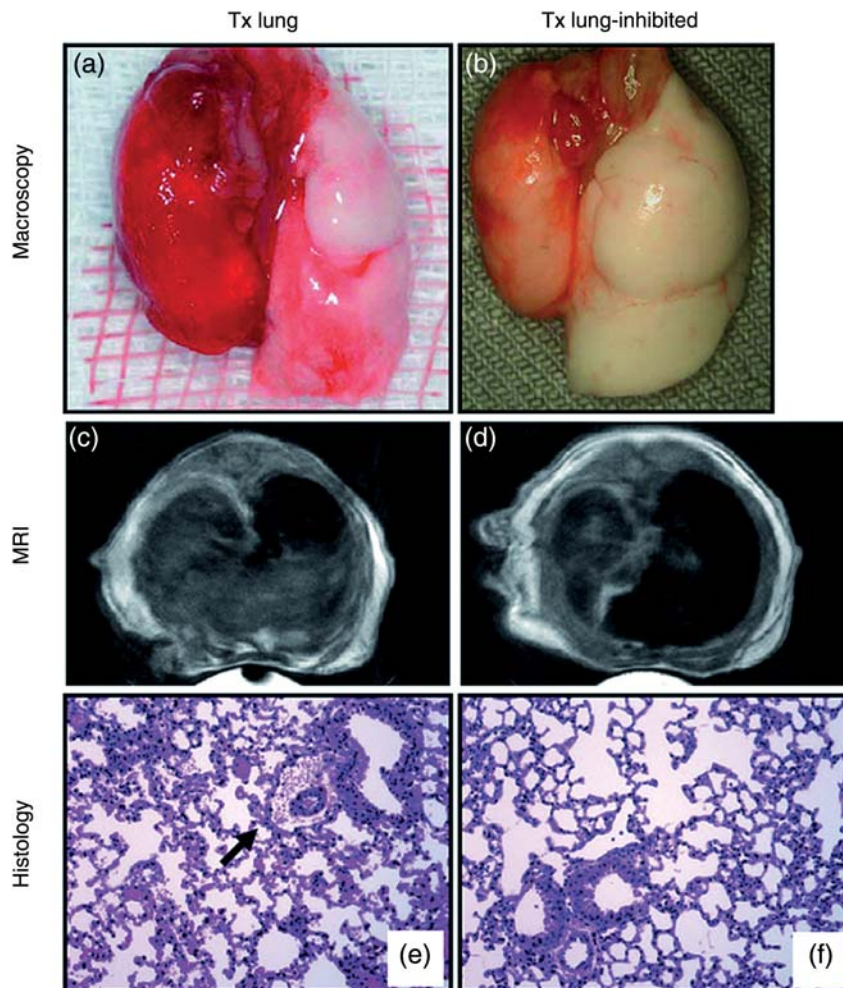


Figure 8: Representative syngrafts from transplanted (Tx) lungs (a) vs. Tx lung from animals that were inhibited (b), macroscopically appeared more inflamed and oedematous (*n* = 4). MR images showed a lower transparency in transplanted (Tx) lungs (c) when compared with Tx lung from animals that were inhibited (d) (*n* = 4). H&E sections from transplanted (Tx) lungs (e) show more perivascular (arrow) and oedema of the alveolar wall (e) when compared with Tx lung from animals that were inhibited (f) (*n* = 6) (magnification: ×100).

hypoxia [21, 22]. It has been shown that SDF-1 gene expression in ischaemic tissue is directly proportional to reduced oxygen tension via upregulation of hypoxia-inducible factor 1 [22]. This

increased SDF-1, which is released from ischaemic endothelial cells, contributes to stem-cell mobilization from bone marrow [23]. Accordingly, we could observe an injury-induced increase

of SDF-1 in Tx lungs that were exposed to 6 h ischaemia time. Systemic DPP-4 inhibition in the recipient led to an even larger increase in SDF-1 levels within the engrafted organ. The lack of DPP-4 enzymatic activity in CD26/DPP-4 KO mice might be the reason for the high SDF-1 concentrations in these animals.

CXCR4, the SDF-1 receptor, was strikingly upregulated in the peripheral blood upon DPP-4 inhibition, which we interpret as an enhanced response to its active ligand SDF-1. A study by Xu *et al.* [24] aimed at reducing cell infiltration upon CXCR4-inhibition in the allogeneic heterotopic model of tracheal implantation in order to lower the inflammatory milieu within the graft. In their study, they found less infiltration by T-cells that were recruited into the graft during chronic rejection. In contrast to allorejection where T-cell plays the major role of host defence, I/R injury primarily relies on infiltrating innate inflammatory cells with subsequent damage to the endothelium [25]. That is why we were particularly interested in endothelial progenitor cells that have regenerative capacity, of which CD34 is the key marker. Malfunction of the engrafted organ is reflected by the induction and release of reactive oxygen species and ischaemia-induced oedema with subsequent swelling of endothelial cells that contributes to damage of the transplant. In fact, when CD34⁺ endothelial progenitor cells were co-stained along with the homing receptor CXCR4 for SDF-1, we found co-expression for both markers on a higher number of cells in Tx-inhibited versus Tx grafts. The presence of the markers CD34 and CXCR4 on CD45⁺ cells in the Tx-inhibited circulation and lungs was highly suggestive of an increased homing into the pulmonary graft. This could also be shown for the tyrosine kinase receptor Flt-3, an early acting factor that supports the survival, proliferation and differentiation of primitive haematopoietic progenitor cells. Taken together, the recruitment of these progenitor cells into the engrafted lung led to a considerable improvement of the histomorphology of the transplant that exhibited less oedema of the alveolar wall and a preserved endothelial lining. However, the exact mechanism by which the enhanced SDF-1–CXCR4 axis is exerting its effect remains largely unclear from our study. Possible explanations include the prevention of apoptosis of endothelial cells or apoptotic endothelial cells being replaced by intact CD34⁺ endothelial cells that were homed to the injured alveolar bed. Another possibility is that niches of progenitor cells with the potency of renewal in the transplanted graft take over the endothelial function. Alternatively, an enhancement of neo-vascularization of the capillary bed leading to reformed intact tissue could occur.

Although we could show a direct correlation between increased SDF-1 levels and enhanced levels of CXCR4, we did not present direct evidence that the inhibition of CD26/DPP-4 with subsequent decreased cleavage and stabilization of intact SDF-1 was responsible for the observed effects. The differentiation between cleaved and uncleaved SDF-1 in biological samples will be part of our future work. Also, the exact mechanism by which the endothelial damage is reduced still has to be further investigated. Finally, we aim to explore whether the observed effect of the enhanced SDF-1–CXCR4 axis can be kinetically followed beyond 48 h, in order to evaluate long-term effects.

The major advantage of the concept presented in this study is the non-invasive approach. Here, regenerative stem cells can be endogenously accumulated by enhancing the SDF-1–CXCR4 axis using a DPP-4 inhibitor. In contrast, conventional stem-cell therapy essentially relies on the exogenous application of regenerative stem cells. Our study surpasses the established clinical

therapeutic concept of the treatment of type II diabetes with DPP-4 inhibitors and the ongoing trials targeting DPP-4/CD26 as a promising therapeutic strategy to stabilize the SDF-1–CXCR4 axis after myocardial ischaemia. We feel that our data encourage further deciphering of the mechanisms finally leading to an improvement of I/R injury after lung transplantation.

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Conflict of interest: none declared.

REFERENCES

- [1] De Meester I, Korom S, Van Damme J, Scharpe S. CD26, let it cut or cut it down. *Immunol Today* 1999;20:367–75.
- [2] Matheeußen V, Baerts L, De Meyer G, De Keulenaer G, Van der Veken P, Augustyns K *et al.* Expression and spatial heterogeneity of dipeptidyl peptidases in endothelial cells of conduct vessels and capillaries. *Biol Chem* 2011;392:189–98.
- [3] Huhn J, Ehrlich S, Fleischer B, von Bonin A. Molecular analysis of CD26-mediated signal transduction in T cells. *Immunol Lett* 2000;72: 127–32.
- [4] Durinx C, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A *et al.* Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur J Biochem* 2000;267:5608–13.
- [5] Zhai W, Jungraithmayr W, De Meester I, Inci I, Augustyns K, Arni S *et al.* Primary graft dysfunction in lung transplantation: the role of CD26/dipeptidylpeptidase IV and vasoactive intestinal peptide. *Transplantation* 2009;87:1140–6.
- [6] Zhai W, Cardell M, De Meester I, Augustyns K, Hillinger S, Inci I *et al.* Intra-graft DPP IV inhibition attenuates post-transplant pulmonary ischemia/reperfusion injury after extended ischemia. *J Heart Lung Transplant* 2007;26:174–80.
- [7] Jungraithmayr W, De Meester I, Matheeußen V, Inci I, Augustyns K, Scharpe S *et al.* Inhibition of CD26/DPP IV attenuates ischemia/reperfusion injury in orthotopic mouse lung transplants: the pivotal role of vasoactive intestinal peptide. *Peptides* 2010;31:585–91.
- [8] Campbell TB, Broxmeyer HE. CD26 inhibition and hematopoiesis: a novel approach to enhance transplantation. *Front Biosci* 2008;13: 1795–805.
- [9] Lapidot T. Mechanism of human stem cell migration and repopulation of NOD/SCID and B2mnull NOD/SCID mice: the role of SDF-1/CXCR4 interactions. *Ann NY Acad Sci* 2001;938:83–95.
- [10] Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M *et al.* Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet* 2003;362: 697–703.
- [11] Ji JF, He BP, Dheen ST, Tay SS. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells* 2004;22:415–27.
- [12] Kollet O, Shvitiel S, Chen YQ, Suriawinata J, Thung SN, Dabeva MD *et al.* HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34⁺ stem cell recruitment to the liver. *J Clin Invest* 2003;112:160–9.

- [13] Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY *et al.* Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest* 2004;114:438–46.
- [14] Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A *et al.* Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med* 2003;198:1391–402.
- [15] Kanki S, Segers VF, Wu W, Kakkar R, Gannon J, Sys SU *et al.* Stromal cell-derived factor-1 retention and cardioprotection for ischemic myocardium. *Circ Heart Fail* 2011;4:509–18.
- [16] Zaruba MM, Theiss HD, Vallaster M, Mehl U, Brunner S, David R *et al.* Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction. *Cell Stem Cell* 2009;4:313–23.
- [17] Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF *et al.* Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci USA* 2000;97:6874–9.
- [18] Jungraithmayr WM, Korom S, Hillinger S, Weder W. A mouse model of orthotopic, single-lung transplantation. *J Thorac Cardiovasc Surg* 2009;137:486–91.
- [19] Scharpe S, De Meester I, Vanhoof G, Hendriks D, van Sande M, Van Camp K *et al.* Assay of dipeptidyl peptidase IV in serum by fluorometry of 4-methoxy-2-naphthylamine. *Clin Chem* 1988;34:2299–301.
- [20] Korom S, De Meester I, Stadlbauer TH, Chandraker A, Schaub M, Sayegh MH *et al.* Inhibition of CD26/dipeptidyl peptidase IV activity *in vivo* prolongs cardiac allograft survival in rat recipients. *Transplantation* 1997;63:1495–500.
- [21] Santiago B, Calonge E, Del Rey MJ, Gutierrez-Canas I, Izquierdo E, Usategui A *et al.* CXCL12 gene expression is upregulated by hypoxia and growth arrest but not by inflammatory cytokines in rheumatoid synovial fibroblasts. *Cytokine* 2011;53:184–90.
- [22] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME *et al.* Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004;10:858–64.
- [23] Farha S, Asosingh K, Xu W, Sharp J, George D, Comhair S *et al.* Hypoxia-inducible factors in human pulmonary arterial hypertension: a link to the intrinsic myeloid abnormalities. *Blood* 2011;117:3485–93.
- [24] Xu J, Torres E, Mora AL, Shim H, Ramirez A, Neujahr D *et al.* Attenuation of obliterative bronchiolitis by a CXCR4 antagonist in the murine heterotopic tracheal transplant model. *J Heart Lung Transplant* 2008;27:1302–10.
- [25] Zhao G, al-Mehdi AB, Fisher AB. Anoxia-reoxygenation versus ischemia in isolated rat lungs. *Am J Physiol* 1997;273:L1112–7.

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EDITORIAL COMMENT

Lung transplantation research: impact of a new surgical model

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Experimental lung transplantation in mice has been deemed 'infeasible' for many years and has been cracked technically only a few years ago by some extraordinarily dedicated researchers. Okazaki and colleagues published the first experimental report based on a series of successful murine left lung transplants in 2007 [1, 2]. While other animal models may more closely resemble the human setting, mouse models have huge advantages when it comes to the study of mechanistic questions on the molecular level. Gene knockout mice have revolutionized whole areas of medical and basic science research and are of no lesser value in transplantation research. Above that, reagents needed for *in vivo* treatment, such as depleting antibodies, proteins or peptides, are easily available in sufficient quantities for mice, but not necessarily for larger animals. The murine heterotopic (non-vascularized) trachea transplantation model was used as an approximation to experimental lung transplantation in mice resembling bronchiolitis obliterans [3], but remains a contraption with severe methodological shortcomings. With the advent of orthotopic mouse lung transplantation, this field of transplantation surgery is now accessible to the modern tools of mechanistic life sciences,

and the study by Jungraithmayr *et al.* [4] in this issue is a good example of this recent development.

CD26, also called dipeptidyl peptidase-4 (DPP4) is a protein expressed on many cell types including lymphocytes and lung parenchyma. It is a membrane glycoprotein with a cell surface epitope acting as an antigen detectable by the respective monoclonal antibodies. Its function is the enzymatic cleavage of certain dipeptides from polypeptides such as growth factors, chemokines and neuropeptides. This degradation inactivates the respective polypeptides. Apparently, CD26/DPP4 cleaves a wide variety of substrates, interfering with many biological pathways including, for example, glucose metabolism and suppression of cancer development. Stromal cell-derived factor 1 (SDF-1, also called CXCL12) is a substrate of CD26/DPP4. Jungraithmayr *et al.* deem SDF-1, together with its receptor CXCR4, important in the modulation of stem cell homing in response to ischaemic injury of the lung. In their experimental setting, they make use of CD26/DPP4 knockout mice, of *in vivo* inhibition of CD26/DPP4 activity using vildagliptin and of a murine orthotopic lung transplantation model to induce ischaemia-reperfusion injury.