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Evidence of an interaction between TGF-β1 and the SDF-1/CXCR4/CXCR7 axis in human platelets

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ABSTRACT

Background: TGF-β1, SDF-1 and its cognate receptors CXCR4 and CXCR7 are expressed on the surface of human platelets and their expression levels are differently regulated in symptomatic coronary artery disease (CAD). All these proteins and receptors influence outcome in patients with symptomatic CAD. There might be a crosstalk between TGF-β1 and the SDF-1/CXCR4/CXCR7 axis. Interrelations in CAD, especially in the context of platelets, are poorly understood. Therefore, we aimed to provide clinical and experimental evidence of interactions between TGF-β1 and the SDF-1/CXCR4/CXCR7 axis in human platelets.

Methods and results: Blood samples of the complete cohort (n = 284) were analysed for platelet surface expression levels of TGF-β1, SDF-1, CXCR4 and CXCR7 by flow cytometry. For stimulation assays platelet rich plasma was treated with TGF-β1 or SDF-1 and then analysed by flow cytometry. Multiple regression analyses were run to show independent associations of TGF-β1 with SDF-1, CXCR4, CXCR7 and clinical cofactors. Both, CXCR4 and CXCR7 significantly predicted TGF-β1 ($p < 0.001$ and $p < 0.001$, respectively). After stimulation with SDF-1, surface expression of TGF-β1 increased significantly when compared to resting platelets [mean TGF-β1 MFI 19.01 *vs.* mean TGF-β1 MFI 14.01, $p < 0.001$]. Upon receptor blocking with either anti-CXCR4 or anti-CXCR7 monoclonal antibodies the enhancing effect of SDF-1 on TGF-β1 surface expression was significantly blunted. Stimulation with TGF-β1 did not alter SDF-1, CXCR4 or CXCR7 expression significantly.

Conclusions: We provide first clinical and experimental data suggesting a cross-talk between TGF-β and the SDF-1/CXCR4/CXCR7 axis in platelets which does not involve transcriptional modulation as shown previously for other cellular systems.

1. Introduction

Platelets are a major source of SDF-1 [1]. Platelets might express and subsequently secrete SDF-1, thereby facilitating chemotaxis and migration of stem cells from bone marrow to peripheral circulation and ultimately to injured tissue [1,2]. Platelet surface expression of SDF-1 is up-regulated in patients with acute coronary syndromes (ACS) as compared to stable coronary artery disease (CAD) [3]. CXCR4 and CXCR7, the two cognate receptors for SDF-1, are expressed on the surface of human platelets [4,5]. Similar to SDF-1, platelet surface expression of CXCR7, but not CXCR4, is elevated in ACS patients and shows significant correlation with surface expression of its ligand [4]. Furthermore, high platelet surface expression of CXCR7 is associated with enhanced functional recovery after ACS [4]. Interestingly however, low platelet CXCR4 levels are associated with increased rate of death and re-infarction in patients with symptomatic CAD [6]. Transforming growth factor beta 1 (TGF-β1) is critically involved in regulatory mechanisms governing cardiac injury, repair and remodelling mechanisms and platelets are one of the major sources of this growth factor [7–9]. Platelet surface expression of TGF-β1 is elevated in ACS patients as compared to patients with stable CAD [10]. In addition, low platelet TGF-β1 is associated with increased mortality and rate of ST-segment elevation myocardial infarction (STEMI) in CAD patients [10]. Recently, several studies have suggested the potential of a functional crosstalk between TGF-β1 and SDF-1 dependent signalling pathways [11,12]. There is evidence, that TGF-β1 up-regulates CXCR4 in several cells and thereby modulates their chemotactic response towards SDF-1 [13]. During the course of tumour progression, TGF-β might lead to enhanced endogenous TGF-β and SDF-1 production and might induce CXCR4 expression in stromal fibroblasts [14]. Recently, it was discovered that SDF-1α significantly stimulated expression of TGF-β in bone marrow derived mesenchymal stem cells (BMSCs). The same group demonstrated that BMSCs might alleviate severe acute pancreatitis and systemic inflammation and might promote tissue repair and angiogenesis by among others up-

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regulating TGF-β. These effects were significantly less distinct when the authors blocked CXCR4 in BMSCs [15]. Another recent study has shown, that TGF-β1 in acute ischemia reperfusion-injured renal tissue markedly increases the CXCR4 surface expression of mesenchymal stem cells (MSCs), possibly contributing to migration of MSCs towards SDF-1 [16]. Furthermore, TGF-β up-regulates CXCR7 in choroid-retinal endothelial cells in a time- and dose-dependent manner [11]. However, interrelations between TGF-β1 and the SDF-1/CXCR4/CXCR7 axis in cardiovascular disease remain poorly understood. Especially in the context of platelets, potential interactions between the afore-mentioned molecules are to the best of our knowledge not deciphered. Hence, the aim of the present study was to evaluate potential associations between TGF-β1 and SDF-1, including its receptors CXCR4 and CXCR7 in platelets of patients with symptomatic CAD. First, we aimed to investigate possible associations in a large cohort of patients with symptomatic CAD. Next, we aimed to provide *in vitro* experimental evidence evaluating the potential mutual influence between these two critical mediators of vascular inflammation and tissue regeneration or remodelling in human platelets (Fig. 1).

2. Subjects and methods

2.1. Patient characteristics and blood sampling

For the cohort study, blood samples were collected during PCI and were analysed for surface expression of TGF-β1, SDF-1, CXCR4 and CXCR7 by flow cytometry within 1 h. All subjects gave written informed consent. Patients were admitted to the department of cardiology at the University Hospital of Tübingen, Germany. We enrolled 284 consecutive patients with symptomatic coronary artery disease (CAD) for analysis. The study was approved by the institutional ethics committee (270/2011BO1) and complies with the declaration of Helsinki and the good clinical practice guidelines [17–19].

2.2. Flow cytometric detection of surface expression of SDF-1, TGF-β1, CXCR4 and CXCR7

Platelets in whole blood were analysed for surface expression of TGF-β1, CXCR4, CXCR7 and SDF-1, gating for the platelet-specific marker CD42b/GPIb. Blood collected in citrate phosphate dextrose adenine (CPDA) anticoagulant was diluted 1:50 with phosphate-buffered saline (PBS; Gibco) and incubated with the respective conjugated antibodies-mouse monoclonal anti-human TGF-β1-PE, mouse monoclonal anti-human CXCR4-PE, mouse monoclonal anti-human CXCR7-PE, mouse monoclonal anti-human SDF-1-CFS (all from R&D systems) and mouse anti-human GPIb-fluorescein isothiocyanate (FITC) (Beckman Coulter) or mouse anti-human GPIb-phycoerythin/PE (BD Biosciences) or their respective isotype controls (mouse IgG1-FITC, mouse IgG2bPE from R&D systems) for 30 min at room temperature (RT). After staining, the samples were fixed with 0.5% paraformaldehyde and analysed by flow cytometry (FACSCalibur flow cytometer Becton-Dickinson, Heidelberg, Germany). For stimulation assays with recombinant human- TGF-β1 (R&D Systems) or recombinant –human/mouse/feline-SDF-1 (R&D Systems), platelet rich plasma (PRP) platelets were isolated from blood of healthy donors as described previously [5]. PRP platelets at 1×10^6/sample were treated with recombinant human- TGF-β1 (1 μg/ml) or recombinant -SDF-1 (5 μg/ml) for 1 h at room temperature and surface expression of TGF-β1, CXCR4, CXCR7 and SDF-1 was evaluated using respective fluorochrome conjugated antibodies and flow cytometric detection as stated before. Blocking of CXCR4 and CXCR7 on platelet surface prior to recombinant SDF-1 treatment was done for 30 min at room temperature where stated using mouse anti-human-CXCR4 (R&D Systems) and mouse anti-human-CXCR7 (R&D Systems), respectively. In the control set mouse IgG was used instead of the blocking antibodies (Fig. 2).

2.3. Statistical analysis

All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago IL). Non-parametric data, including median fluorescence intensities (MFIs), were compared using the Mann–Whitney *U* test. Correlations were assessed by Spearman's rank correlation coefficient (ρ). Multiple linear regression analyses were performed to evaluate independent associations between TGF-β1 and SDF-1, CXCR4, CXCR7, co-medication and clinical factors (Fig. 3). Data were transformed using natural logarithm to optimize for linearity. Non-parametric MFIs are presented as median values and 25th and 75th percentiles. Since we investigated more than 2 groups for the SDF-1 stimulation experiments, one-way ANOVA with Tukey post-hoc correction was used to analyse the results. These MFIs are presented as mean values with mean differences and 95% CI. MFIs in Fig. 4 are presented as mean with 95% CI.

3. Results

Patient characteristics of the 284 consecutive patients included in the cohort are presented in Table 1.

3.1. Platelet surface expression of TGF-β1 correlates with platelet SDF-1, CXCR4 and CXCR7 in patients with symptomatic CAD

We were able to provide TGF-β1 levels for n = 259 patients, CXCR4 levels for n = 284, CXCR7 levels for n = 276 and SDF-1 levels for n =

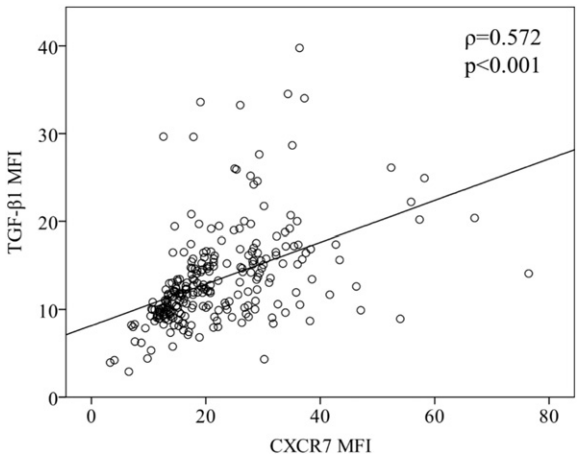

Fig. 1. Correlation between TGF-β1 MFI and CXCR7 MFI using Spearman rank correlation coefficient (ρ).

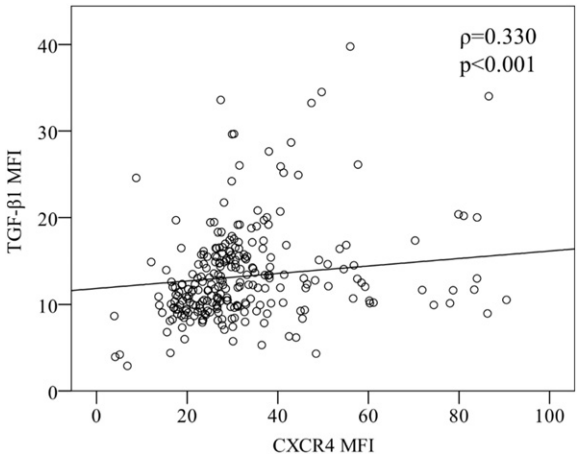

Fig. 2. Correlation between TGF-β1 MFI and CXCR4 MFI using Spearman rank correlation coefficient (ρ).