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Short communication

Endothelial microparticle-promoted inhibition of vascular remodeling is abrogated under hyperglycaemic conditions

Felix Jansen*,[1], Andreas Zietzer[1], Tobias Stumpf, Anna Flender, Theresa Schmitz, Georg Nickenig, Nikos Werner*

Department of Internal Medicine II, Rheinische Friedrich-Wilhelms University, Bonn, Germany

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ABSTRACT

Background: Endothelial microparticles (EMPs) inhibit vascular remodeling by transferring functional microRNA (miRNA) into target vascular smooth muscle cells (VSMCs). Because EMPs are increased in diabetic patients and potentially linked to vascular complications in diabetes mellitus, we sought to determine whether effects of EMPs generated under high glucose concentration on vascular remodeling might differ from EMPs derived from untreated cells.

Methods and results: EMPs were generated from human coronary endothelial cells (HCAEC) exposed to high glucose concentrations in order to mimic diabetic conditions. These EMPs were defined as 'hyperglycaemic' EMPs (hgEMPs) and their miRNA transfer capacity and functional effects were compared with EMPs generated from 'healthy' untreated HCAECs. In vitro, the intercellular transfer of antiproliferative miRNA-126-3p from ECs to VSMCs via EMPs was significantly reduced under hyperglycaemic conditions. Additionally, EMP-mediated inhibition of the miRNA-126-3p target LRP6 and of VSMC migration and proliferation was abrogated, when hgEMPs were used. In vivo, the inhibitory effect of EMPs on neointima formation, VSMC proliferation and macrophage infiltration was abolished in mice treated with hgEMPs.

Conclusion: Pathological hyperglycaemic conditions weaken potentially protective intercellular communication mechanisms by affecting EMP content and function.

1. Introduction

Diabetes mellitus represents a major risk factor for cardiovascular morbidity and mortality. As a consequence of endothelial activation and dysfunction, diabetic patients show increased plasma levels of circulating endothelial cell-derived microparticles (EMPs) [1]. Endothelial microparticles transfer microRNA (miRNA) to various cells and affect thereby the phenotype of the recipient cell [2]. Recent studies revealed, that diabetes mellitus additionally affects circulating MP-incorporated miRNA expression patterns [3,4]. In this context, the expression level of vasculoprotective miRNA-126-3p was significantly reduced in EMPs in experimental and clinical hyperglycaemic conditions [4,5].

We recently demonstrated that a reduced miRNA-126-3p expression in circulating MPs was associated with a significantly increased rate of coronary revascularizations, which represents a clinical "model" of neointima formation in response to coronary stenting or in progressive atherosclerotic disease development [6]. In particular after stent implantation, coronary artery stenosis development is triggered by exceeding VSMC proliferation.

Diabetic patients have an augmented risk to develop neointimal hyperplasia requiring repeat coronary interventions. Whereas EMPs from untreated cells display an inhibitory effect on vascular remodeling [6], the influence of hyperglycaemic conditions on EMP-mediated intercellular communication route, however, is unknown.

Here, we present evidence that EMPs derived under pathological hyperglycaemic conditions in vitro show a reduced antiproliferative, vasculoprotective function compared to EMPs from untreated cells.

2. Methods

Methods are described in detail in the Online supplements.

3. Results and discussion

As hgEMPs express significantly lower levels of miRNA-126-3p

* Corresponding authors at: Medizinische Klinik und Poliklinik II, Universitätsklinikum Bonn, Sigmund-Freud-Str. 25, Bonn 53127, Germany.
E-mail addresses: felix.jansen@ukbonn.de (F. Jansen), nikos.werner@ukbonn.de (N. Werner).
[1] These authors contributed equally.

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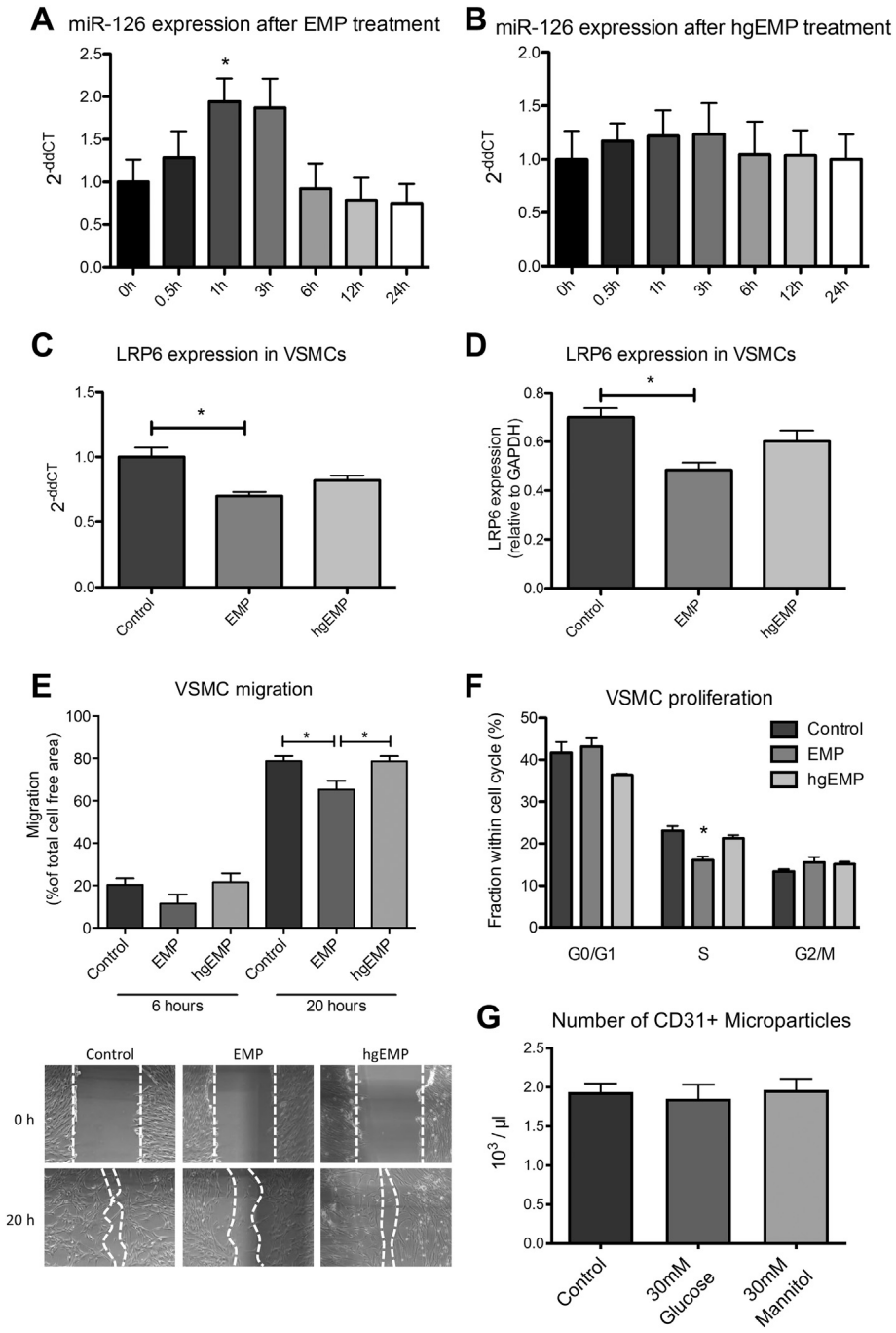

Fig. 1. Influence of EMPs and hgEMPs on VSMC proliferation in vitro.
A,B) VSMCs were treated for different time periods with EMPs, hgEMPs or vehicle. TaqMan qPCR analysis of miRNA-126 expression in VSMCs. RNU6 was used as endogenous control (*$p < 0.05$, n = 6–8). C,D) miRNA-126 target LRP6 expression in EMP-, hgEMP- and untreated VSMCs was determined by qPCR and western blot (*$p < 0.05$, n = 6–8). E) Confluent VSMCs in basal media were co-incubated with EMPs, hgEMPs or vehicle. Scratch assay was performed and representative images of cells migrating and proliferating into the scratched region are shown. Quantitative analysis of migration/proliferation was measured as percentage of total cell-free area (*$p < 0.05$, n = 10–12). F) Proliferation of VSMCs was measured by flow cytometric bromodeoxyuridine (BrdU) incorporation analysis (*$p < 0.05$, n = 7–9). G) EMP release of HCAECs was assessed after glucose and mannitol treatment via flow cytometry (n = 8).

compared to EMPs [5], we first explored whether this results in a reduced intercellular transfer capacity of intravesicular miRNA-126-3p into VSMCs. After EMP treatment of VSMCs, qPCR experiments revealed a time-dependent increase of endothelial miRNA-126-3p expression in VSMCs, which was lacking after hgEMP treatment (Fig. 1A,B). Accordingly, EMP-mediated inhibition of miRNA-126-3p target LRP6 was abolished in hgEMP-treated VSMCs on mRNA and protein level (Fig. 1C,D). As LRP6 promotes VSMC migration and proliferation [7], we next explored whether the reduced inhibitory effect of hgEMPs on LRP6 expression results in different functional consequences of hgEMPs compared to EMPs. Indeed, scratch assay and BrdU incorporation experiments displayed an inhibitory effect of EMPs on migration and proliferation, which was abrogated after hgEMP treatment (Fig. 1E,F). To ensure EMPs and hgEMPs were administered in comparable concentrations, EMP secretion of HCAEC was assessed under different conditions via flow cytometry. No significant influence of prior glucose or mannitol treatment on CD31 + EMP secretion was detected (Fig. 1G).

In patients, increased VSMC migration and proliferation fosters neointimal hyperplasia resulting in arterial narrowing or occlusion [8]. Arterial intimal hyperplasia contributes to in-stent restenosis and atherosclerotic lesion development. As increasing evidence suggests that paracrine signaling mechanisms are crucially involved in the regulation of vascular remodeling processes [9], we next tested the impact of hgEMPs and EMPs on neointima formation in mice and their miRNA transfer capacity in vivo. In line with in vitro data, EMP, but not hgEMP treatment, increased miRNA-126 expression in target vessels (Fig. 2A). Furthermore, immunhistochemical and immunofluorescent stainings showed an inhibitory effect of EMPs on neointima formation, VSMC proliferation and macrophage infiltration in vivo, which was abolished