FEBS Letters xxx (2014) xxx–xxx

journal homepage: www.FEBSLetters.org

Shedding of dipeptidyl peptidase 4 is mediated by metalloproteases and up-regulated by hypoxia in human adipocytes and smooth muscle cells

Diana Röhrborn [a], Jürgen Eckel [a,b], Henrike Sell [a,*]

[a] Paul-Langerhans-Group for Integrative Physiology, German Diabetes Center, Düsseldorf, Germany
[b] German Center for Diabetes Research (DZD e.V.), Düsseldorf, Germany

ARTICLE INFO

Article history:
Received 20 May 2014
Revised 13 August 2014
Accepted 27 August 2014
Available online xxxx

Edited by Laszlo Nagy

Keywords:
CD26/DPP4
Hypoxia
Non-classical secretion
Protease

ABSTRACT

Dipeptidyl peptidase 4 is an important drug target for diabetes and a novel adipokine. However, it is unknown how soluble DPP4 (sDPP4) is cleaved from the cell membrane and released into the circulation. We show here that MMP1, MMP2 and MMP14 are involved in DPP4 shedding from human vascular smooth muscle cells (SMC) and MMP9 from adipocytes. Hypoxia increased DPP4 shedding from SMC which is associated with increased mRNA expression of MMP1. Our data suggest that constitutive as well as hypoxia-induced DPP4 shedding occurs due to a complex interplay between different MMPs in cell type-specific manner.

1. Introduction

Dipeptidyl peptidase 4 (DPP4) is a glycoprotein of 110 kDa, which is ubiquitously expressed on different cell types. The extracellular part of this type II cell surface protein is substantially glycosylated, which plays an important role in the interaction with different proteins [1]. As an exopeptidase of the serine protease type, DPP4 cleaves numerous substrates at the penultimate position and thereby mostly inactivates them. Among these are peptides (e.g. stromal cell-derived factor 1 alpha (SDF1α), eotaxin) and cytokines (*monocyte chemotactic protein-1* (MCP-1), interleukin 2 (IL-2)) as well as the incretin hormones [1]. The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are major regulators of the postprandial insulin release [2]. Therefore gliptins, a class of specific DPP4 inhibitors, are now widely used as a monotherapy or combination therapy for type 2 diabetes. By inhibition of DPP4 activity, these drugs prolong the half-life of GLP-1 and GIP which then stimulate pancreatic insulin secretion, suppress glucagon production and thereby contribute to an improved glycemic control [2,3].

DPP4 is not only present on the surface of cells, but can also be found in the circulation [4]. By comprehensive proteomic profiling of the adipocyte secretome, we could identify soluble DPP4 (sDPP4) as a novel adipokine, with an upregulated release throughout the differentiation of adipocytes [5,6]. We confirmed that mature adipocytes in comparison to macrophages and preadipocytes are a major source of DPP4 [5]. Elevated serum levels of DPP4 were found in obese patients and correlate with the size of adipocytes and risk factors for the metabolic syndrome.

It has been reported, that DPP4 as a type II transmembrane protein is cleaved of the cell membrane in a process called shedding [7]. The nature of enzymes contributing to the shedding of DPP4 and the regulation of this process is largely unknown. The aims of our study were to elucidate the underlying shedding mechanism and to explore the regulation of sDPP4 release in vitro. We show here for the first time that members of the metalloprotease family are involved in the release of sDPP4 from different primary human cells.

Abbreviations: ADAM, a disintegrin and metalloprotease; bref A, brefeldin A; DPP4, Dipeptidyl peptidase 4; sDPP4, soluble dipeptidyl peptidase 4; MMP, matrix metalloprotease; SMC, human vascular smooth muscle cell; TACE, tumor necrosis factor α converting enzyme

* Corresponding author at: Paul-Langerhans-Group for Integrative Physiology, German Diabetes Center, Auf'm Hennekamp 65, D-40225 Düsseldorf, Germany. Fax: +49 211 3382697.
E-mail address: henrike.sell@ddz.uni-duesseldorf.de (H. Sell).

http://dx.doi.org/10.1016/j.febslet.2014.08.029
0014-5793/

2. Materials and methods

2.1. Materials

Complete protease inhibitor (04693116001), and PhosStop phosphatase (04906837001) inhibitor cocktail were provided by Roche. Reagents for SDS–PAGE were supplied by Amersham Pharmacia Biotech and by Sigma. DPP4 rabbit polyclonal antibody (H00001803-D01P) was obtained from Abnova. Beta actin mouse antibody (ab6276) was supplied by Abcam. HRP-conjugated goat anti-rabbit (W4011) and goat anti-mouse (W4021) IgG antibodies where purchased from Promega.

Collagenase NB4 (17465.02) was obtained from Serva. FCS (10270-106), Dulbecco's modified Eagles/HAM F12 (DMEM/F12) medium (42400-010), α-modified Eagle's (α MEM) medium (11900-016) and Ham's F-12 medium (21700-026) was supplied by Gibco (Invitrogen). Troglitazone was obtained from Sigma Aldrich.

The Protease inhibitors AEBSF (ALX-270-022), E64 (ALX-260-007) were dissolved in water. BB-94 (196440), MMP9 Inhibitor I (444278) and MMP2 Inhibitor III (444288) were purchased from Calbiochem and dissolved in sterile DMSO. Human Protease Array Kit (ARY021) was obtained from R&D Systems.

2.2. Adipocyte isolation and culture

Preadipocytes of human subcutaneous adipose tissue were obtained from lean or moderately overweight subjects undergoing plastic surgery. Isolation was performed as previously described [8]. Cells from up to passage 4 were grown to confluence in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12) supplemented with 10% FCS with medium change every 2-3 days. Passaging of the preadipocytes was performed according to Skurk et al. [9]. Differentiation was started by adding 5 μmol/l troglitazone for 3 days to adipocyte differentiation medium (DMEM/F12, 33 μmol/l biotin, 17 μmol/l D-panthothenic-acid, 66 nM insulin, 1 nM triiodo-L-thyronine, 100 nM cortisol, 10 μg/ml apo-transferrin, 50 μg/μl gentamycin, 15 mmol/l HEPES, 14 nmol/l $NaHCO_3$, pH 7.4) as described previously [5]. After 14 days cells where treated with the indicated substances diluted in α-modified DMEM and incubated for the indicated periods.

2.3. Smooth muscle cell culture

Primary human smooth muscle cells (SMC) from three different donors (Caucasian, one male, two females) were supplied as proliferating cells from Lonza, TebuBio and PromoCell and kept in culture according to the manufacturer's protocol. For all experiments subconfluent cells of passage three were used. SMC were characterized by morphologic criteria and by immunostaining with smooth muscle α-actin. For the experiments 100.000 cells/ml were seeded and grown for 24 hours in Growth medium (Promocell) with appropriate supplements. After washing with PBS and serum starvation for 24 hours cells were treated as indicated.

2.4. Hypoxia

For hypoxic experiments, cells were exposed to 1% O_2 supplemented with 5% CO_2 and respective concentrations of nitrogen in an Xvivo hypoxia chamber system (Biospherix) for 24 or 48 h.

2.5. ELISA

DPP4 release to the cell culture medium was measured by human DPP4 DuoSet ELISA (R&D Systems, DY1180) according to the manufacturer's instructions.

2.6. Silencing of target genes

SMC were seeded in 6-well plates and grown until 60–80% confluence. Silencing experiments were performed by using 40 nM FlexiTube siRNA (Qiagen, MMP1 (SI03021802), MMP14 (SI03648841)) and 12 μl HiPerfect (Qiagen, 301705) according to the manufacturers' instructions. At day 10-12 of differentiation adipocytes were treated with 40 nM of the respective siRNA and 9 μl of HiPerfect. Optimal transfection conditions were tested by separate titration experiments. To control for unspecific effects, control cells were treated with AllStars Negative Control siRNA (QIAGEN, 1027280). After 24 h mRNA was isolated to check for silencing efficiency. Supernatants were collected after 24 or 48 h respectively.

2.7. qRT-PCR

Total RNA was isolated and reverse transcribed using the RNeasy Mini Kit (Qiagen, 74106) and Omniscript Reverse Transcription kit (Qiagen, 205113) according to the manufacturer's instructions. Gene expression was determined by quantitative real-time PCR (qRT PCR) using QuantiTect primer assays (Qiagen, ACTB, QT00057428, QT00001533, QT00040040, QT00088396, QT00014581, QT00055580) and GoTaq qPCR Master Mix (Promega, A6002) with 0.04-0.4 ng of generated cDNA on a Step One Plus Cycler (Applied Biosystems). Beta-actin was used as a reference gene and expression levels of investigated genes were normalized to beta-actin. Gene expression was analyzed via the ΔΔCt method and compared with the designated control.

2.8. Statistical analysis

Data are expressed as mean ± SEM. Unpaired two-tailed Student's *t* test or one-way ANOVA (post hoc test: Bonferroni's multiple comparison test) were used to determine statistical significance. All statistical analysis was done using Prism (GraphPad, La Jolla, CA, USA) considering a *P* value of less than 0.05 as statistically significant. Corresponding significance levels are indicated in the figures.

3. Results

3.1. sDPP4 release is insensitive to brefeldin A treatment

In accordance with previously published data on sDPP4 release from skeletal muscle cells [10], sDPP4 release did not follow the classical ER/Golgi-dependant pathway in SMC and adipocytes, because it was insensitive to brefeldin A (bref A) treatment (Fig. 1A and B). The applied concentrations of bref A were effective in blocking IL-6 secretion from SMC (Fig. 1A) and adiponectin secretion from adipocytes (Fig. 1B).

3.2. Protease profile differs between SMC and adipocytes

To find out what types of proteases are released from different cell types and if there is a difference in the release profile of proteases, we used supernatants of adipocytes and SMC in a Protease Profiler Array. This array enables us to assess the release of 34 different proteases of the four main types of proteases, namely matrix metalloproteases (MMP), serine proteases, cysteine proteases and aspartyl proteases. A huge panel of proteases spotted on this membrane belongs to the Cathepsins, which are serine, cysteine or aspartyl proteases. The comparison of different members of the Cathepsin family showed, that the detectable subtypes were more or less identical in the different cell types (Data not shown). In

Please cite this article in press as: Röhrborn, D., et al. Shedding of dipeptidyl peptidase 4 is mediated by metalloproteases and up-regulated by hypoxia in human adipocytes and smooth muscle cells. FEBS Lett. (2014), http://dx.doi.org/10.1016/j.febslet.2014.08.029