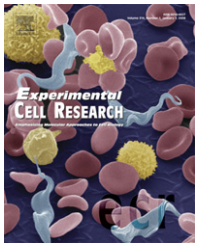
EXPERIMENTAL CELL RESEARCH 314 (2008) 2501–2514

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

MT1-MMP releases latent TGF-β1 from endothelial cell extracellular matrix via proteolytic processing of LTBP-1

*Olga Tatti, Piia Vehviläinen, Kaisa Lehti, Jorma Keski-Oja**

Departments of Pathology and Virology, University of Helsinki and Helsinki University Hospital, 00014 Helsinki, Finland

ARTICLE INFORMATION

Article Chronology:
Received 7 March 2008
Revised version received
26 May 2008
Accepted 27 May 2008
Available online 6 June 2008

Keywords:
LTBP
ECM
TGF-β
MT1-MMP
Endothelial cells

ABSTRACT

Targeting of transforming growth factor beta (TGF-β) to the extracellular matrix (ECM) by latent TGF-β binding proteins (LTBPs) regulates the availability of TGF-β for interactions with endothelial cells during their quiescence and activation. However, the mechanisms which release TGF-β complexes from the ECM need elucidation. We find here that morphological activation of endothelial cells by phorbol 12-myristate 13-acetate (PMA) resulted in membrane-type 1 matrix metalloproteinase (MT1-MMP) -mediated solubilization of latent TGF-β complexes from the ECM by proteolytic processing of LTBP-1. These processes required the activities of PKC and ERK1/2 signaling pathways and were coupled with markedly increased MT1-MMP expression. The functional role of MT1-MMP in LTBP-1 release was demonstrated by gene silencing using lentiviral short-hairpin RNA as well as by the inhibition with tissue inhibitors of metalloproteinases, TIMP-2 and TIMP-3. Negligible effects of TIMP-1 and uPA/plasmin system inhibitors indicated that secreted MMPs or uPA/plasmin system did not contribute to the release of LTBP-1. Current results identify MT1-MMP-mediated proteolytic processing of ECM-bound LTBP-1 as a mechanism to release latent TGF-β from the subendothelial matrix.

Introduction

The targeting of transforming growth factor beta (TGF-β) to the extracellular matrix (ECM) by latent TGF-β binding proteins (LTBPs) regulates the availability and activation of TGF-β during various cellular processes including endothelial cell activation, migration and apoptosis [1,2]. Inactivating mutations in TGF-β receptors and signaling pathways account for a number of vascular pathologies [3–5], but recent findings show that also mutations in extracellular matrix proteins, which regulate the availability of the active TGF-β in vascular environment, result in pathological conditions, such as the Marfan syndrome and hypertension [6–9]. Regulation of TGF-β concentration is crucial also for balancing the activation and resolution phases in

* *Corresponding author*. University of Helsinki, Biomedicum/A506b, P.O. Box 63, Haartmaninkatu 8, 00014 Helsinki, Finland.
E-mail address: Jorma.Keski-Oja@helsinki.fi (J. Keski-Oja).

Abbreviations: ADAM, a disintegrin and metalloproteinase; BSA, bovine serum albumin; ECs, endothelial cells; ECM, extracellular matrix; ERK, extracellular signal-regulated protein kinase; HUVEC, human umbilical vein endothelial cells; LAP, latency associated peptide; LLC, large latent TGF-βcomplex; LTBP, latent TGF-β binding protein; MEK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MT-MMP, membrane-type matrix metalloproteinase; PBS, phosphate buffered saline; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PI-3-kinase, phosphoinositide 3-OH kinase; SLC, small latent TGF-βcomplex; TGF-β1, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinase; uPA, urokinase-type plasminogen activator

0014-4827/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2008.05.018

angiogenesis. In early steps low TGF-β levels promote endothelial cell proliferation and migration, whereas during the maturation phase higher levels of active TGF-β induce the integrity of the vessel wall, the recruitment and differentiation of smooth muscle cells and the deposition of extracellular matrix [10–12]. In addition, increased levels of TGF-β induce tumor progression and metastasis through the stimulation of angiogenesis [13–15].

Like most non-malignant cell types, endothelial cells secrete TGF-βs as biologically inactive complexes, which consist of the mature growth factor and its latency associated propeptide (LAP) forming the small latent TGF-β complex (SLC). Most of the SLC produced by endothelial cells is covalently bound to an LTBP forming the large latent TGF-β complex (LLC) [16]. TGF-β can be efficiently secreted only in association with LTBPs [17]. After secretion LTBP attaches covalently with the subendothelial ECM, thus targeting TGF-β to ECM [18]. LTBPs are high molecular weight ECM proteins that belong to the fibrillin/LTBP superfamily which, in addition to their role in biology of TGF-β, are structural components of the elastic ECM [19–21]. Three of LTBP isoforms, namely LTBP-1, 3 and 4, bind latent TGF-β [22]. Cleavage of LTBPs releases latent TGF-β from the matrix [23,24]. Following the release TGF-β can be activated through either proteolytic processing or conformational change of LAP [25]. The release of ECM-bound latent TGF-β occurs during various tissue remodeling events, such as angiogenesis, inflammation and wound repair, and is seen as one of the regulatory steps to control TGF-β activity and signaling [26].

To date, the suggested proteolytic mechanisms to regulate the bioavailability of TGF-β in endothelial cells have been established using recombinant enzymes, such as human mast cell chymase and leukocyte elastase, which can release latent TGF-β1 from subendothelial matrix by cleaving LTBP-1 [16]. During apoptosis of endothelial cells, serine proteases release ECM-bound TGF-β via processing the LLC [27]. In co-cultures of endothelial cells with smooth muscle cells, latent TGF-β can be activated on the surface of smooth muscle cells, presumably by plasmin [28]. In fibroblastic cells plasmin, thrombin and also bone morphogenic protein-1 (BMP1) can liberate latent TGF-β from the ECM [23,29]. In addition, plasmin and MMP-2-mediated degradation of LTBP-1 has been proposed as a physiological mechanism for the release of latent TGF-β from the matrix of osteoclasts [24]. Given the recognized role of ECM as an important regulator of TGF-β functions [9,18,26], we focused this study on exploring endogenous mechanisms, which regulate the availability of TGF-β by releasing latent TGF-β from the ECM of cultured endothelial cells. Interestingly, we found that during endothelial cell activation both free LTBP-1 and LLC were released in truncated forms by the membrane-anchored matrix metalloproteinase, MT1-MMP. The proteolytic processing coincided with markedly increased expression of MT1-MMP, and could be selectively inhibited by the metalloproteinase inhibitors that block MT-MMP activity and by shRNA-mediated knockdown of MT1-MMP.

Materials and methods

Antibodies and reagents

Mouse monoclonal antibody against human LTBP-1 (mab388) and goat polyclonal antibody against β1-LAP (AF-246-NA) were purchased from R&D Systems. Polyclonal rabbit antibody against human LTBP-1 (Ab39) was a kind gift of Dr. C.-H. Heldin (Ludwig Institute for Cancer Research, Uppsala, Sweden). Rabbit polyclonal anti-LTBP-2 antibodies were as described [30]. Rabbit polyclonal anti-fibronectin antibody was from Sigma, goat anti-collagen IV and rabbit anti-laminin antibody from Chemicon. Affinity-purified rabbit anti-human TGF-β1-LAP (680) was used as described by Taipale [18]. Anti-ERK1/2 rabbit polyclonal antibody was from Promega. Phospho-p44/42 MAP kinase rabbit polyclonal antibody was from Cell Signaling. TAPI-1 and GM6001 (MMP and ADAM inhibitors), AEBSF (serine protease inhibitor), LY294002 (phosphatidylinositol 3-kinase (PI-3K)-inhibitor), bisindoylmaleimide-1 (protein kinase C inhibitor), PD98059 were from Calbiochem. DRB (CK-II inhibitor) was from Biomol International LP. Aprotinin (serine protease inhibitor) was purchased from Sigma. UO126 (MEK kinase inhibitor) was from Promega.

Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from PromoCell. They were cultured in endothelial cell growth medium MV containing 5% of fetal calf serum (PromoCell). Cells were maintained at seeding ratio 1:3 and used for the experiments between passages 4–7.

Immunofluorescence analysis

Cells were grown on glass coverslips for 4 d after reaching confluency in the endothelial cell growth medium MV containing 5% of fetal calf serum (PromoCell) and then treated with different substances in the same medium. During extended culture to facilitate the formation of ECM (up to 6 d), the cell culture medium was changed every 2 d. Coverslips were then washed with phosphate buffered saline (PBS) and the cells fixed with 3% paraformaldehyde (PFA) at 4 °C for 10 min. After washing three times with PBS, the cells were incubated in PBS containing 5% bovine serum albumin (BSA) to prevent non-specific binding of the antibodies. The cells were then incubated with the primary antibody in Dulbecco's PBS for 1 h. The bound antibodies were detected using Alexa Fluor conjugated secondary antibodies (Invitrogen). The coverslips were finally briefly washed with distilled H_2O, mounted on glass slides using Vectashield anti-fading reagent (Vector Laboratories) and examined under the Axioplan 2 imaging microscope (Zeiss) using a 20× objective. Images were acquired with AxioCamHRc camera (Zeiss) and AxioVision3.1 software (Zeiss).

Isolation and plasmin digestion of ECM-associated proteins

After reaching confluency, HUVECs were cultured on 6-well plates for 4 d, treated with PMA and the indicated inhibitors for 24 h, and sodium deoxycholate insoluble matrices were isolated and digested with plasmin to solubilize LTBPs [18]. Briefly, the cell cultures were washed once with PBS and extracted by 0.5% sodium deoxycholate in 10 mM Tris–HCl buffer, pH 8.0, containing 150 mM NaCl and 1% NP-40, and clarified by centrifugation at 8000 ×*g* for 10 min. The sodium deoxycholate insoluble material was then washed once with ice-cold PBS and digested with 0.3 U/ml of plasmin (Sigma) in PBS containing 1 mM $MgCl_2$, 1 mM