Available online at www.sciencedirect.com
ScienceDirect
The International Journal of Biochemistry & Cell Biology 40 (2008) 1581–1596
IJBCB
www.elsevier.com/locate/biocel

Elastokine-mediated up-regulation of MT1-MMP is triggered by nitric oxide in endothelial cells

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Received 25 September 2007; received in revised form 30 November 2007; accepted 30 November 2007
Available online 8 December 2007

Abstract

Membrane-type I matrix metalloproteinase (MT1-MMP) has been previously reported to be up-regulated in human microvascular endothelial cell-1 line (HMEC) by elastin-derived peptides (elastokines). The aim of the present study was to identify the signaling pathways responsible for this effect. We showed that elastokines such as $(VGVAPG)_3$ peptide and kappa elastin induced nitric oxide (NO) production in a time-, concentration- and receptor-dependent manner as it could be abolished by lactose and a receptor-derived competitive peptide. As evidenced by the use of NO synthase inhibitors, elastokine-mediated up-regulation of MT1-MMP and pseudotube formation on Matrigel required NO production through activation of the PI_3-kinase/Akt/NO synthase and NO/cGMP/Erk1/2 pathways. Elastokines induced both PI_3-kinase p110γ sub-unit, Akt and Erk1/2 activation, as shown by a transient increase in phospho-Akt and phospho-Erk1/2, reaching a maximum after 5 and 15 min incubation, respectively. Inhibitors of PI_3-kinase and MEK1/2 suppressed elastokine-mediated MT1-MMP expression at both the mRNA and protein levels, and decreased the ability of elastokines to accelerate pseudotube formation. Besides, elastokines mediated a time- and concentration-dependent increase of cGMP, suggesting a link between NO and MT1-MMP expression. This was validated by the use of a guanylyl cyclase inhibitor, a NO donor and a cGMP analog. The guanylyl cyclase inhibitor abolished the stimulatory effect of elastokines on MT1-MMP expression. Inversely, the cGMP analog, mimicked the effect of both elastokines and NO donor in a concentration- and time-dependent manner. Overall, our results demonstrated that such elastokine properties through NO and MT1-MMP may be of importance in the context of tumour progression.

Keywords: Endothelial cells; Elastokines; Nitric oxide; MT1-MMP; Signaling pathway

Abbreviations: MMP, matrix metalloproteinase; (p)MT1-MMP, (pro)membrane-type I matrix metalloproteinase; (p)MMP2, (pro)matrix metalloproteinase-2; HMEC, human microvascular endothelial cell-1 line; KE, kappa elastin; NO, nitric oxide; NOS, NO synthase; VEGF, vascular endothelial growth factor; ECM, extracellular matrix; ECGM MV, endothelial cell growth medium MV; HRP, horseradish peroxidase; ECGS/H, endothelial cell growth supplement/heparin; FCS, fetal calf serum; V-14, VVGSPSAQDEASPL peptide; V-18, $(VGVAPG)_3$ peptide; PIP_3, phosphatidyl-inositol-3 phosphate; sGC, soluble guanylyl cyclase.

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1357-2725/$ – see front matter
doi:10.1016/j.biocel.2007.11.022

1. Introduction

Matrix metalloproteinases (MMPs) belong to the family of zinc-dependent enzymes that were originally described as matrix-degrading enzymes, i.e. matrixins (Visse & Nagase, 2003). It is now recognized that their repertoire of substrates is not restricted to extracellular matrix components but also encompasses other molecules such as cytokines, growth factors, serpins, cell adhesion molecules, endocytosis receptors and tissue transglutaminase (Mc Cawley & Matrisian, 2001; Nagase, Visse, & Murphy, 2006). Thus, MMPs participate to the regulation of the activity of many of those molecules and also to the activation of proteolytic cascades. Consequently, MMPs appear to play a crucial role in a myriad of physiological and pathological processes, particularly in tumour growth, invasion and metastasis through modification of cancer cells microenvironment (Egeblad & Werb, 2002; Nelson, Fingleton, Rothenberg, & Matrisian, 2000). The pivotal importance of Membrane-type matrix metalloproteinases (MT-MMPs) in directing pericellular proteolysis and subsequent cell invasion is now well documented (Sounni & Noel, 2005). Among that MMP subfamily, MT1-MMP was described to play an essential function in melanoma progression and angiogenesis (Hofmann, Houben, Bröcker, & Becker, 2005; Neitzel, Craig, Magee, & Malafa, 1999;Seiki, 2003; Seiki & Yana, 2003). MT1-MMP was initially identified on the surface of invasive lung carcinoma cells (Sato et al., 1994), but is also expressed in several other cell types, including endothelial cells where it is assumed to direct their migration and tubulogenesis in vitro and in vivo (Seiki, 2003).

Much evidence indicated that extracellular matrix (ECM) was a key regulator of MT1-MMP expression; for instance, MT1-MMP up-regulation by invasive melanoma cells and endothelial cells in three-dimensional type I collagen lattice was demonstrated (Galvez, Matias-Roman, Albar, Sanchez-Madrid, & Arroyo, 2001; Hass, Davis, & Madri, 1998; Lafleur, Handsley, Kaupner, Murphy, & Edward, 2002; Löffek et al., 2006; Ntayi, Lorimier, Berthier-Vergnes, Hornebeck, & Bernard, 2001). Moreover, several extracellular matrix-derived peptides designated as matrikines, particularly those derived from elastin designated as elastokines which possess a GxxPG consensus sequence (Bellon, Martiny, & Robinet, 2004; Maquart, Bellon, Pasco, & Monboisse, 2005), were shown to modulate MT1-MMP expression and activation further influencing melanoma growth and invasiveness (Ntayi et al., 2004). Conspicuous degradation of fibrous elastin is one hallmark of melanoma progression being associated with higher stage of disease, lymph nodes occurrence and distant metastasis (Feinmesser et al., 2002). We previously reported that binding of locally generated elastokines to M3Da melanoma cells could enhance their MT1-MMP-mediated invasiveness through a collagen matrix (Ntayi et al., 2004). More recently, in keeping with vascular involvement in melanoma, elastokines, i.e. kappa elastin, a soluble form of elastin obtained by alkaline hydrolysis of the insoluble polymer (Jacob & Hornebeck, 1985), and synthetic peptides possessing the VGVAPG motif found as repeats in tropoelastin, proved to display potent proangiogenic property in vitro and in vivo. Such an effect on angiogenesis was attributed solely to MT1-MMP up-regulation by elastokines (Robinet et al., 2005). These effects were shown to be mediated by the binding of elastokines to a 67-kDa multifunctional high affinity receptor with lectin-like properties designated as EBP for elastin binding protein (Hinek, 1994; Mecham et al., 1989). EBP was further identified as an inactive spliced form of β-galactosidase known as S-Gal (Hinek, Rabinovitch, Keeley, Okamura-Oho, & Callahan, 1993; Privitera, Prody, Callahan, & Hinek, 1998).

Our aim was to delineate the mechanisms involved in elastokine-mediated MT1-MMP expression and angiogenic phenotype in microvascular endothelial cells (HMECs). Experimental approaches were guided by previous reports showing that (i) elastokines could trigger in vivo an endothelium-dependent vasorelaxation involving both nitric oxide (NO) synthase (NOS) and cyclooxygenase pathways (Faury, Garnier et al., 1998; Faury, Usson, Robert-Nicoud, Robert, & Verdetti, 1998), (ii) NO could stimulate the expression of several MMPs from different cell types in culture. For instance, NO was described to increase MMP-9 expression in cultured rat vascular smooth muscle cells (Marcet-Palacios et al., 2003), rabbit articular chondrocytes (Sasaki et al., 1998), human trophoblast cells (Novaro et al., 2001), and endothelial cells (Steinle et al., 2002). Other reports also demonstrated an up-regulation of MMP-2 expression by NO (Hirai et al., 2001; Novaro et al., 2002; Steinle et al., 2002). More recently, NO was shown to induce MMP-13 expression and activity in bovine aortic endothelial cells (Zaragoza, Balbin, Lopez-Otin, & Lamas, 2002; Zaragoza, Soria et al., 2002) and reciprocally, MMP-13 was demonstrated to be a downstream effector of NO-activated endothelial cell migration (Lopez-Rivera et al., 2005). However, other reports mentioned a NO-mediated down regulation of MMPs (Chen & Wang, 2004; Eagleton et al., 2002; Eberhardt et al., 2000; Gurjar, Sharla, & Bhalle, 1999; Phillips & Birnby, 2004). Besides its role in vascular tone (Moncada, Radomski,