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Abeta(1–40)-induced secretion of matrix metalloproteinase-9 results in sAPPα release by association with cell surface APP

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To understand matrix metalloproteinase-9 (MMP-9) involvement in Alzheimer's disease, we examined mechanisms mediating increased expression of MMP-9 in the presence of Abeta(1–40) and the role of MMP-9 on amyloid precursor protein (APP) processing. Up-regulation of MMP-9 expressed by SK-N-SH cells in the presence of Aβ (1–40) was mediated by $\alpha_3\beta_1$ and $\alpha_2\beta_1$ integrin receptors. Overexpression of MMP-9 or treatment of HEK/APP695 cells with activated recombinant MMP-9 resulted in enhanced secretion of soluble APP (sAPPα), a product of α-secretase cleavage, and reduction of Aβ release. MMP-9 effect was enhanced by phorbol 12-mysistrate-13-acetate (PMA), an α-secretase activator and inhibited by EDTA or SB-3CT, an MMP-9 inhibitor. Additionally, immunoprecipitation and confocal microscopy demonstrated that MMP-9 and APP695 were associated on the cell surface. These results indicate that Aβ peptide increases MMP-9 secretion through integrins; MMP-9 then directly processes cell surface APP695 with an α-secretase like activity, substantially reducing the levels of secreted Aβ peptide.

Keywords: MMP-9; Amyloid precursor protein; Secretase; Integrin; Alzheimer; Amyloid beta peptide

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of brain amyloid plaques, composed mostly of amyloid beta peptides (Aβ40–42) (Selkoe, 2001), generated by proteolytic processing of amyloid precursor protein (APP) (Goldgaber et al., 1987; Robakis et al., 1987).

Cleavage of APP by α-secretases within the Aβ sequence results in the secretion of a soluble N-terminal APP fragment (sAPPα) and generation of a membrane-bound COOH terminal fragment (CTF). This processing does not result in Aβ formation (Selkoe et al., 1988; Esch et al., 1990; Anderson et al., 1991). However, cleavage by β- and γ-secretases generates Aβ peptides and an ectodomain derivative (sAPPβ) (Estus et al., 1992; Haass et al., 1992; Seubert et al., 1993).

Two subclasses of the metalloproteinase family of proteases, matrix metalloproteinases (MMPs) and their close relatives a disintegrin and metalloprotease (ADAMs) have been implicated in receptor shedding including APP (Werb, 1997; Tanaka et al., 2004; Ahmad et al., 2006). MDC9, ADAM 10 and TACE, members of the ADAM family of proteases, have been shown to have α-secretase activity (Buxbaum et al., 1998; Koike et al., 1999; Lammich et al., 1999). Collagenases/Matrix metalloproteinases (MMPs) are a family of zinc-metalloproteinases which, among others, degrade extracellular matrix (ECM) proteins in normal and pathological conditions (Werb, 1997; Nagase and Woessner, 1999). In the central nervous system MMPs are synthesized by astrocytes, microglia and neurons (Gottschall and Deb, 1996; Asahina et al., 2001). Elevated levels of MMPs have been reported in the cortex and hippocampus of AD patients relative to control individuals (Leake et al., 2000). In the hippocampus of AD patients, increased immunoreactivity of the inactive form of MMP-9 was observed around the plaques (Backstrom et al., 1992; Backstrom et al., 1996), whereas a very recent report stated that activated astrocytes surrounding amyloid plaques showed enhanced expression of MMP-2 and MMP-9 in aged APP/presinilin 1 mice (Yin et al., 2006). These observations have correlated increased expression of MMP-9 with AD. Furthermore, active matrix metalloproteinase-9 (MMP-9) has been reported to degrade in vitro synthetic Aβ(1–40) at the α-secretase cleavage site between *Lys 16-Leu 17* and amyloid beta fibrils (Backstrom et al., 1996; Yan et al., 2006). Expression of MMP-9 is mediated at least in part by signals transmitted inside the cells

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via integrins (Larjava et al. 1993; Agrez et al. 1999; DiPersio et al. 2000). Additionally, β1-containing integrins were shown to mediate adhesion to Aβ(1–40) via the amino acid sequence arg-his-asp (RHD), which resembles the RGD integrin-binding sequence (Ghiso et al. 1992).

To provide evidence regarding the possible role of MMP-9 in AD, we examined mechanisms mediating increased expression of MMP-9 in the presence of Abeta(1–40) and the role of MMP-9 on APP processing. Using human neuroblastoma cells we present evidence that α3β1 and α2β1 integrin receptors modulate the expression of MMP-9 following cell binding to Aβ (1–40) substrate.

Using HEK-293 cells stably transfected with APP695, we obtained data indicating that both transfection-induced and recombinant/4-aminophenylmercuric acetate (APMA)-activated MMP-9 resulted in increased constitutive and receptor-regulated sAPPα release as well as decreased secretion of Aβ(1–40). Processing of APP by MMP-9 should take place on the cell surface, since intracellular MMP-9 is inactive and additionally APP695 was found to interact with MMP-9 on the cell surface.

Materials and methods

Cell cultures and transient transfection

Human neuroblastoma SK-N-SH cells (ATCC, Rockville, MD, USA) were cultured in minimum essential medium (Eagle) supplemented with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 1.5 g/l sodium bicarbonate, 10% FBS and antibiotics (all from Biochrom Seromed, Berlin, Germany). To estimate the expression of MMP-9 in the presence of soluble Aβ(1–40) peptide, cells were cultured for 24 h in fresh serum-free media containing 10 μM soluble Aβ(1–40) peptide. Conditioned media free of cell debris were supplemented with 1 mM Na_2EDTA and 0.02% sodium and stored at −80 °C. Control cells were cultured in the absence of Aβ(1–40).

Human embryonic kidney cells (HEK-293), wild type or stably expressing APP695 were grown in DMEM supplemented with 10% FBS and 4 mM L-glutamine. Stably transfected cells were maintained in medium containing 0.8 mg/ml geneticin (GIBCO-BRL, Gaithersburg, MD, USA).

The pcDNA3.1Myc 6His/pro-MMP-9 construct encoding the full length of human pro-MMP-9 was a kind gift from Dr. Rafael Fridman (Department of Pathology, Wayne State University School of Medicine, Karmanos Cancer Institute, Detroit, Michigan, USA). HEK-293 cells stably expressing APP695 (HEK/APP695) were transiently transfected with the cDNA of MMP-9 (HEK/APP695/MMP-9) with the use of lipofectamine 2000 (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's protocol. 24 h post-transfection, the medium was replaced with fresh serum-free medium and cells were cultured for another 24 h. Conditioned media free of cell debris were stored at −80 °C until further use. For protein kinase C (PKC) activation, 1 μM phorbol 12-mysistrate-13-acetate (PMA) (Sigma St. Louis, Missouri, USA) in 0.01% DMSO (Sigma) was added in culture 4 h before collecting the conditioned media (Caporaso et al., 1992).

Antibodies and reagents

Monoclonal antibodies against integrins [α3 (P1B5), β1 (P5D2), α2 (P1E6) and αvβ3 (LM609)], polyclonal antibodies Ab805 and Ab16996 against MMP-9, monoclonal antibodies 6E10 and 4G8 against sAPPα and Ab respectively (as well as intact APP), and MMP-9 inhibitor SB-3CT were all purchased from Chemicon International (Temecula, California, USA). Sources of other commercial antibodies were anti-β-tubulin (Sigma), anti-6-His tag 27E8 (Cell Signaling Technology, Beverly, MA USA); fluorescein-conjugated IgGs (Cappel, ICN Pharmaceuticals, Frankfurt, Germany), rhodamine-conjugated IgGs (Santa Cruz Biotechnology, California, USA), and peroxidase-conjugated secondary antibodies (Amersham Biosciences, Uppsala, Sweden). Human recombinant APMA-activated MMP-9 was obtained from Calbiochem (La Jolla, CA, USA). The Aβ(1–40) peptide was synthesized by solid-phase method. Its purity and structure were confirmed by HPLC and mass spectroscopy analysis respectively. For water-free storage to prevent aggregation of Aβ into its fibrillar form, the peptide was treated as previously described (Matter et al., 1998).

Zymography

Gelatin zymography was performed as previously described (Anderson et al. 1996). Briefly, aliquots of conditioned media were subjected to SDS-PAGE under non-reducing conditions in 10% polyacrylamide gels containing 0.1% gelatin. The volume of conditioned medium loaded per lane was adjusted according to the cell number obtained at harvest. After electrophoresis, SDS was removed from the gel by washing in 50 mM Tris–HCl pH 7.5, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 2.5% Triton X-100 and 0.02% NaN_3 and the gel was incubated in the same buffer without Triton X-100 for 48 h at 37 °C. Following staining with Coomassie Brilliant Blue R-250 for 3 h and de-staining in water, gelatin-degrading enzymes were identified by their ability to clear the gelatinous substrate at their respective molecular mass.

To examine the effect of integrins in MMP-9 secretion, cells were pre-incubated with saturated concentrations of anti-integrin monoclonal antibodies (40 μg/ml each and 0.5 μg/ml anti-β1) for 30 min at 37 °C, diluted 10-fold and cultured for 24 h. Control cells were cultured in the absence of antibodies. The medium was replaced with fresh serum-free medium containing monoclonal antibodies as stated above, and cells were allowed in culture for another 24 h. Conditioned media were collected for zymography.

Immunoblotting

Electrophoresis in the presence of SDS was performed on total cell lysates or conditioned media, according to the method of Laemmli (1970) on 10% polyacrylamide gels under reducing conditions. Cells were lysed in PBS pH 7.4 containing 1 mM PMSF, 1 mM NEM, 1% Triton X-100, 1 mM $CaCl_2$ and a cocktail of protease inhibitors and the protein concentration was determined by the Bradford method (Pierce). The resolved proteins were subsequently transferred to Hybond-ECL nitrocellulose membrane (Amersham) by electroblotting (Towbin et al., 1979). Blots were saturated for 1 h at room temperature with 5% non-fat milk in TBS, 0.1% Tween-20 and incubated overnight at 4 °C with the appropriate dilutions of antibodies, in the same buffer without Tween-20. The membranes were incubated for 1 h at room temperature with peroxidase (HRP)-conjugated secondary antibodies and for the detection the ECL detection system by Amersham was followed.