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Neuroprotective role of MMP-9 overexpression in the brain of Alzheimer's 5xFAD mice

Apostolia Fragkouli, Effie C. Tsilibary, Athina K. Tzinia *

Laboratory of Cell & Matrix Pathobiology, Institute of Biology, NCSR Demokritos, Athens, Greece

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ABSTRACT

Accumulation of amyloid-β (Aβ) peptide is believed to play a central role in the pathogenesis of Alzheimer's disease (AD). Lowering Aβ levels in the brain may thus improve synaptic and cognitive deficits observed in AD patients. In the non-amyloidogenic pathway, the amyloid-β precursor protein (APP) is cleaved within the Aβ peptide sequence by α-secretases, giving rise to the potent neurotrophic N-terminal fragment sAPPα. We have previously reported that gelatinase B/matrix metalloproteinase 9 (MMP-9), a matrix metalloproteinase critically involved in neuronal plasticity, acts as α-secretase both *in vitro* and *in vivo* and reduces Aβ levels *in vitro*. In the present study, we demonstrate that neuronal overexpression of MMP-9 in a transgenic AD mouse model harboring five familial AD-related mutations (5xFAD) resulted in increased sAPPα levels and decreased Aβ oligomers without affecting amyloid plaque load in the brain. Functionally, overexpression of MMP-9 prevented the cognitive deficits displayed by 5xFAD mice, an improvement that was accompanied by increased levels of the pre-synaptic protein synaptophysin and mature brain-derived neurotrophic factor (BDNF) in the brain. These results suggest that *in vivo* activation of endogenous MMP-9 could be a promising target for interference with development and/or progression of AD.

Introduction

Alzheimer's disease (AD), the most prevalent age-related neurodegenerative disorder, is characterized by widespread synaptic loss, neuronal death and progressive cognitive decline. One of the hallmarks of AD is the abnormal deposition of aggregated amyloid-β peptide (Aβ) in extracellular plaques within the brain. Aβ, generated upon cleavage of the parent amyloid-β precursor protein (APP) by β- and γ-secretases, can form soluble oligomers, that were reported to be neurotoxic (Cheng et al., 2007; Dodart et al., 2002; Lacor et al., 2007; Mucke et al., 2000; Selkoe, 2002; Walsh and Selkoe, 2007; Walsh et al., 2002), as well as large insoluble aggregates, the major components of mature plaques, which do not appear linked with toxicity (Lee et al., 2007). Alternatively, APP can be cleaved within the Aβ sequence by enzymes with α-secretase activity, giving rise to the N-terminal fragment soluble APPα (sAPPα), a potent neurotrophic factor (Turner et al., 2003), that has been shown to play a critical role in neuronal plasticity and memory formation (Bour et al., 2004; Ring et al., 2007; Taylor et al., 2008).

Shift of APP processing in favor of the β- and γ-secretase pathway appears to be a key event in the pathogenesis of AD; it has been shown that β-secretase activity is increased and α-secretase activity is decreased in post-mortem tissue from sporadic AD patients (Tyler et al., 2002). Consistently, it has been reported that sAPPα levels are decreased in the cerebrospinal fluid (CSF) of patients with either familial (Lannfelt et al., 1995) or sporadic (Colciaghi et al., 2002; Fellgiebel et al., 2009; Lannfelt et al., 1995) forms of AD and most importantly decreased sAPPα levels in the CSF were correlated with impaired cognitive function (Almkvist et al., 1997; Fellgiebel et al., 2009). The above findings collectively support the concept that decreased sAPPα levels could explain, independently of Aβ changes, some of the cognitive deficits observed in AD, and thus provide additional support for the concept of enhancing α-secretase activity, as a mean to interfere with AD development and progression.

Converging evidence during the past decades has demonstrated that several proteases, including matrix metalloproteinases (MMPs), act cooperatively in different cellular compartments to regulate brain Aβ steady-state level and may thus play a crucial role in the pathogenesis of AD. MMPs constitute a family of more than 20 zinc-dependent proteases, mostly secreted as inactive pro-enzymes that are activated upon proteolytic cleavage (Nagase and Woessner, 1999) and are able to degrade extracellular matrix, as well as cell-surface and other proteins (Cauwe et al., 2007; Yong et al., 2001). In the nervous system, MMPs appear to participate in a variety of physiological processes including axonal outgrowth, myelination and plasticity (Agrawal et al., 2008; Yong et al., 2001), as well as in a number of pathological conditions, such as neuroinflammation and epiloptogenesis (Agrawal et al., 2008; Konnecke and Bechmann, 2013; Mizoguchi and Yamada, 2013).

* Corresponding author at: Institute of Biology, National Centre for Scientific Research "Demokritos", 15310 Agia Paraskevi, Athens, Greece. Fax: +30 210 6511 767.
E-mail address: atzin@bio.demokritos.gr (A.K. Tzinia).
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With respect to AD, several MMPs have been implicated in APP proteolytic processing, such as the membrane bound MT1-MMP and MT3-MMP that have been shown to cleave APP *in vitro* leading to different than those described above APP fragments (for review see Cauwe et al., 2007). Probably the most well-studied MMPs are the two members of the gelatinase subfamily, gelatinase A (matrix metalloproteinase-2, MMP-2) and gelatinase B (matrix metalloproteinase-9, MMP-9). In animal models of AD, MMP-2 and MMP-9 have been shown to be overexpressed in reactive astrocytes surrounding amyloid plaques (Yan et al., 2006) and studies using genetic or pharmacological inhibition of MMP-2 or MMP-9 activity have further indicated that amyloid deposition is increased when gelatinase activity is decreased (Ridnour et al., 2012; Yin et al., 2006). Although data relating MMP-2 or MMP-9 expression with AD in humans appear to be rather controversial reporting not only reduced, but also unchanged levels of MMP-2 and MMP-9 in the CSF from AD patients (Baig et al., 2008; Hanzel et al., 2014; Horstmann et al., 2010; Mroczko et al., 2014), several studies have established that these metalloproteinases are able to degrade soluble Aβ both *in vitro* and *in vivo* (Backstrom et al., 1996; Roher et al., 1994; Yan et al., 2006; Yin et al., 2006). Finally, and in contrast to various proteases involved in APP processing, MMP-9 has been shown to be the only Aβ-degrading enzyme capable of also degrading Aβ fibrils *in vitro* and Aβ plaques *in situ* (Backstrom et al., 1996; Yan et al., 2006).

We have previously reported that MMP-9 is also involved in receptor-mediated sAPPα release *in vitro* and that it exhibits α-secretase-like activity *in vivo* (Fragkouli et al., 2011, 2012; Talamagas et al., 2007). Transgenic mice overexpressing MMP-9 in brain neurons (TgMMP-9) display increased brain levels of sAPPα, as well as enhanced neuronal plasticity and cognitive performance (Fragkouli et al., 2012); nevertheless, it remains unclear whether increased expression or activity of MMP-9 in the brain interferes with the AD-related pathology. In order to address this question, we have crossed TgMMP-9 mice with transgenic mice harboring familial AD (FAD)-related mutations in APP and presenilin-1 (5xFAD) (Oakley et al., 2006). Herein, we report that neuronal overexpression of MMP-9 resulted in increased levels of sAPPα and alleviated the formation of Aβ oligomers without affecting the plaque burden or astrocyte activation in the brain of 5xFAD mice. Moreover, our behavioral analysis revealed that overexpression of MMP-9 restored the cognitive abilities of 5xFAD mice and this improvement was accompanied by increased levels of the synaptic protein synaptophysin and enhanced maturation of the brain-derived neurotrophic factor (BDNF).

Materials and methods

Experimental animals

Generation of 5xFAD and TgMMP-9 mice was previously described; 5xFAD mice co-express and co-inherit FAD mutant forms of human APP [Swedish (K670N, M671L), Florida (I716V) and London (V717I) mutation] and presenilin-1 (M146L; L286V) transgenes under the transcriptional control of the neuron-specific mouse Thy-1 promoter (Oakley et al., 2006) and TgMMP-9 mice express the wild-type human pro-MMP-9 sequence under the transcriptional control of the neuron-specific promoter platelet-derived growth factor (PDGF)-β (Fragkouli et al., 2012). 5xFAD (Tg6799 line) and TgMMP-9 mice were both kept in a heterozygote state in a C57BL/6 background and for the generation of 5xFAD/TgMMP-9 mice, TgMMP-9 females (backcrossed into C57BL/6 background for 5 generations) were crossed with 5xFAD males (backcrossed into C57BL/6 background for more than 10 generations). All animals were kept under standard conditions (24 °C, 12-h light/dark cycle, lights on at 8:00 AM), housed in mixed genotype groups and received food and water *ad libitum*. Given the preponderance of AD in women (Fratiglioni et al., 1997; Jorm et al., 1987; Tang et al., 1996), in the present study we focused our analysis on female animals; our choice was further supported by pilot experiments revealing a significant sex difference on MMP-9 potential gelatinolytic activity among 5xFAD/TgMMP-9 animals, with females displaying an approximately 50% increase in the brain (see Supplemental Fig. 1). Care was taken to minimize the number of animals used, as well as their suffering. All animal experimentations were carried out in agreement with the ethical recommendations of the European Communities Council Directive of 22 September 2010 (2010/63/EU).

Behavioral studies

Twenty four female and 24 male animals (eight wild-type, eight 5xFAD and eight 5xFAD/TgMMP-9), all littermates aged 6 1/2 months old at the onset of the behavioral studies, were used. All mice were housed in mixed genotype groups of three to four and were habituated to the experimenter during 5-minute handling sessions over 3 consecutive days prior to behavioral testing. Mice were tested in three behavioral tasks in the following order: spontaneous alternation T-maze, novel object recognition and water-maze task. During behavioral testing females were not monitored for estrous cycle phase, in order to avoid subjecting them to the stress of taking vaginal smears.

Spontaneous alternation T-maze

The T-maze apparatus we used (two arms perpendicular to a central stem), as well as the protocol we followed (one forced 30-second trial followed by ten 30-second free choice trials on the same day, with a 15-minute inter-trial interval) have been previously described (Fragkouli et al., 2005). Consecutive choices made by the mice were recorded and alternation rate during 10 free-choice trials was calculated.

Novel object recognition

On the next day, mice were tested in the novel object recognition task and the protocol we used has been previously described (Fragkouli et al., 2012). Briefly, it consisted of a habituation session (one 5-minute trial in the absence of objects over three consecutive days), a familiarization session on the next day (three 5-minute trials, with a 15-minute inter-trial interval in the presence of two identical objects) and a probe trial (one 5-minute trial in the presence of one new and one familiar object). Time spent exploring each object (exploration time) during the probe trial was scored and in order to avoid confounding effects of possible differences in exploratory behavior among animals, discrimination ratio (time spent exploring the novel object-time spent exploring the familiar object / total exploration time) was also calculated.

Water-maze

On the following day, mice were tested in the water-maze task. The apparatus and the protocol used have been previously described (Fragkouli et al., 2012). In brief, the protocol we followed consisted of a 2-day visible phase (four 60-second trials per day in the presence of a movable visible platform), a 5-day acquisition phase (four 60-second trials per day in the presence of a hidden platform, located in a fixed position relative to visible extramaze cues) and a 60-second probe trial one day after the acquisition phase (in the absence of the platform, starting from the same position, opposite to the target quadrant i.e. the quadrant where the platform was located during acquisition). The behavior of animals during the water-maze was analyzed by the behavioral analysis system Etho Vision 1.96 (Noldus). In order to assess learning in the visible and the acquisition phase of the task, two parameters were analyzed; latency (time in seconds to reach the platform) and swim path length (in meters). The values obtained were averaged per mouse within each daily session. In the probe trial, time spent in each of the pool quadrants was analyzed and time spent in the target quadrant was used as a parameter of memory. To ensure that behavior in the water-maze did not simply reflect changes in activity, swim speed