Matrix metalloproteinase-9 in glutamate-dependent adult brain function and dysfunction

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Matrix metalloproteinase-9 (MMP-9) is extracellularly operating protease that is expressed by the adult brain neurons and released in response to enhanced neuronal activity driven by glutamate. In addition, MMP-9 can also be produced by glial and endothelial cells as well as by infiltrating leukocytes. Extracellularly, the protein is activated in a cascade of steps that involves also other proteases and then is inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs). Under physiological conditions, MMP-9 is involved in neuronal plasticity, including long-term potentiation as well as learning and memory. This function of MMP-9 may relate to its synaptic action that is a consequence of post-synaptic/dendritic locus of mRNA and protein storage and release. Under pathological conditions that involve excessive function of glutamate, such as excitotoxicity, stroke and traumatic brain injury, MMP-9 is detrimental to the brain tissue, probably because of its excessive activity. This complex functionality and dysfunctionality of MMP-9 is well documented by studies on its expression patterns as well as with an aid of chemical inhibitors and knockout mice.

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent endopeptidase that together with the most closely related MMP-2, forms a subfamily of gelatinases.[1,2] MMPs operate extracellularly (predominantly secreted pericellularly, and some membrane-bound) and are locally inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs) that bind MMPs non-covalently.[1]

MMPs are expressed as inactive zymogens in which the cysteine residue in the propeptide binds to Zn^{2+} present at the active site of the enzyme. Activation occurs when interaction between cysteine and Zn^{2+} is disrupted, mainly by cleavage the propeptide off.[1,3] The activation of the proenzyme is controlled by a cascade of steps involving other MMPs and plasmin system (Figure 1). When activated, the MMP-9 can be inhibited by TIMPs, particularly by TIMP-1.[1,4] Notably, due to all the regulatory mechanisms, such as secretion, interaction with membrane receptors, internalization and TIMP-1 inhibition, MMP-9 acts outside the cell only focally and transiently to prevent excessive activity.[5]

Even though there are multiple substrates of MMP-9 identified *in vitro*, only a few of them have been confirmed *in vivo*.[2] In the brain, the list includes laminin, β-dystroglycan, zonae occludens-1 and myelin basic protein as well as NG2 proteoglycan in the spinal cord.[6–9]

MMP-9 is expressed ubiquitously, albeit at low levels, in various mammalian organs and tissues, including the adult brain[10,11] where it is produced mainly by neurons, and, to some extent, by glia.[12–15] Recently, we studied a subcellular localization of MMP-9 in the rat hippocampus, using high-resolution morphological as well as biochemical approaches.[16] At the ultrastructural level, MMP-9 was found to be present in a subset of dendritic spines bearing asymmetric (e.g. glutamatergic) synapses. The presence of MMP-9 in spines was reported also in the cerebellum,[13] whereas in neuronal culture MMP-9 was detected in the growth cones.[17]

MMP-9 and Neuronal Plasticity

The synaptic localization of MMP-9 implies its role in neuronal plasticity. Indeed, the physiological role of MMP-9 has been supported by functional studies involving MMP-9 KO mice as well as chemical inhibitors and application of TIMP-1. In particular, Nagy *et al.*[18] reported for MMP-9 KO mice a deficiency in late phase of long-term potentiation (L-LTP), a well-established model of synaptic plasticity, as well as in memory tested in a fear conditioning paradigm. Moreover, the inhibition of MMP-9 using either antisense oligonucleotides or a broad-specificity MMP inhibitor was reported to impair hippocampal learning in Morris water maze in rats.[19] Recently, Okulski *et al.*[20] reported that blocking MMP-9 by specific chemical inhibitor or by overexpression of TIMP-1 impairs late phase of LTP in the prefrontal cortex in freely moving rats and cortical slices *in vitro*. It is also of note that TIMP-1/MMP-9 system comprises a target for AP-1 transcription factor, whose role in neuronal plasticity, learning and memory is well documented (see Jaworski *et al.*[21] and Kaczmarek *et al.*[22]). Electrically or chemically evoked seizures are often used as an experimental paradigm to study cellular and molecular mechanisms of plasticity. Kainic acid is an agonist of ionotropic glutamate receptors.[23] When injected intraperitoneally, it causes seizures that could last even for several hours.[24] This seizure activity results in the death of pyramidal neurons in the CA subfield of hippocampus, the cells in the limbic (including entorhinal) cortex and the amygdala within 24–72 h after the neurotoxin administration.[24–26] In result, the granule neurons of DG, lose their CA3 targets as well as they lose their entorhinal cortex inputs and in consequence, they undergo strong, albeit aberrant neuronal plasticity to create new connections.[25] This plasticity involves first an elimination of dendritic spines, and then subsequent sprouting of granule cells axons. Importantly, the sprouting is followed by autaptic synaptogenesis on the granule cells dendrites, thus forming a

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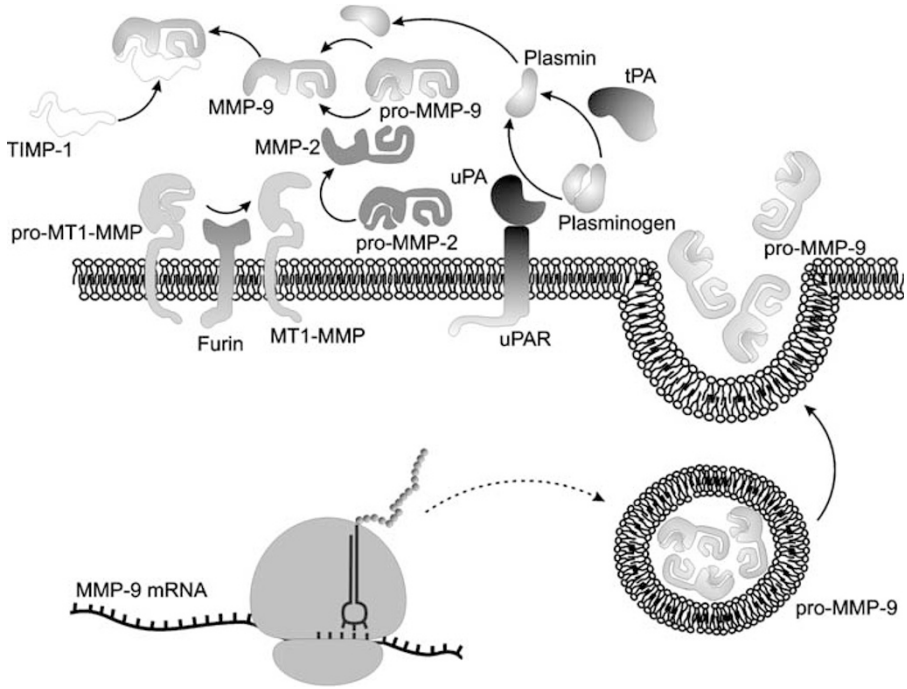

Figure 1 Schematic representation of MMP-9 synthesis and activation. MMP-9 mRNA translocated toward the dendrites is locally translated with the protein eventually localized to secretory vesicles. In response to glutamate-driven neuronal activity, MMP-9 is released outside the cell, where through the cascade of proteolytic steps (involving, e.g., furin, MT1-MMP, MMP-2, tPA, uPA and plasmin) is transformed from the latent to the active form. Once activated, MMP-9 is quickly inactivated by interactions with its inhibitor, TIMP-1. TIMP-1, tissue inhibitor of matrix metalloproteinases-1; MMP, matrix metalloproteinase; MT, membrane type; tPA, tissue type plasminogen activator; uPA, urokinase type plasminogen activator; uPAR, uPAR receptor

recurrent excitatory connections that are believed to be an anatomical substrate of a remote post-kainate epilepsy.[27]

Increased MMP-9 activity following the kainate was reported by Zhang *et al*.[28] and Szklarczyk *et al*.,[14] who have found that the plasticity occurring in the dentate gyrus is spatially and temporally correlated with prolonged increases in MMP-9 expression and activity. Using electron microscopic *in situ* hybridization, and quantitative RT-PCR assay on synaptosomal mRNA, we have recently found that MMP-9 mRNA increase occurred even in the most distal branches of the dendritic tree, including spines very close to postsynaptic membranes (Konopacki *et al*., submitted). Thus, it seems that MMP-9 mRNA undergoes an activity-dependent dendritic transport that serves local protein expression at active synapses. It is of note that the phenomenon of the dendritic mRNA translocation is recognized as being one of the most fundamental mechanisms underlying synaptic plasticity.[29] Regarding the functional aspects, our recent studies indicate that at the early period after the kainate administration, MMP-9 is involved in the elimination of dendritic spines and then is also required for the aberrant synaptogenesis on the granule cell dendritic tree that is provided by those cells' own axons (Wilczynski *et al*., submitted). Incidentally, it might suggest that MMP-9 contributes to a development of epileptic foci based in aberrant synaptogenesis.

MMP-9 in Neuronal Death

Apart from its physiological role(s) in the brain, MMP-9 appears to be also engaged in numerous pathological processes in the CNS, many of which are associated with glutamate dysfunction. The most obvious example is excitotoxicity, which occurs if glutamate receptor activation becomes excessive or prolonged, leading to the point where the target neurons become damaged and eventually die, for example, in the CA1 and CA3 hippocampal subfields after the kainate[25,26] and indeed using kainate treatment of the organotypic hippocampal cultures Jourquin *et al*.[15] showed that MMP-9 is directly involved in the excitotoxic neuronal loss. Furthermore, overexpression of TIMP-1 in such cultures showed neuroprotective effect after excessive glutamate stimulation.[30]

Excitotoxicity is also present as a secondary effect after traumatic brain injury. It was shown in both most popular models of that disorder (controlled cortical impact and corticectomy) that already at 3 h post-injury, MMP-9 activity was increased and remained elevated even until 7 days.[11,31,32] Importantly, MMP-9 KO mice were found to be more resistant to traumatic brain injury, since they had smaller post-traumatic lesions after the head trauma, and recovered motor skills faster.[32]

Another disorder of CNS in which MMP-9 plays a vital role is ischemia. It is either an absolute or relative shortage in the blood supply. If the ischemic conditions are maintained in the brain, they result in neuronal loss that involves multiple neuronal death pathways. For instance, transient global cerebral ischemia that may arise, for example, from cardiac arrest or severe hypotension, induces selective, delayed neuronal death, which is limited to some of brain regions, including CA1 of the hippocampus. CA1 neurodegeneration is accompanied by activation of microglia and astrocytes and their invasion to the sites of neurons death.[33] The level of

MMP-9 was reported to increase significantly at 3 days after the ischemia and reached a peak at 6 days.[34,35] Unfortunately, the studies on localization of gelatinase activity after ischemia are inconsistent. According to Rivera *et al.*[34] immunoreactivity of MMP-9 decreased in neurons of the hippocampus of ischemic animals, 24 h after reperfusion and increased outside the cell bodies as well as in association with cells scattered in the CA1 subfield. Three days after the ischemia, MMP-9 staining was present mostly in microglial cells with some immunoreactivity associated with neurons in CA1 subfield, and 6 days after the ischemia MMP-9 was observed almost exclusively in astroglia. Other studies showed, however, that MMP-9 and its gelatinolytic activity 3 days after ischemia was present mainly in neurons and only to some extent in astrocytes.[35,36] Never the less, also in this condition, MMP-9 KO mice and treatment with MMP inhibitor reduced the neuronal damage.[35]

In contrast to the global ischemia model, in focal ischemia, lesions develop already within 12 h.[33] Furthermore, in this case, ischemic injury appears to be caused mainly by necrosis, and only in the later stages, if the appropriate energy state of tissue is maintained, may also be caused by apoptosis.[33] Notably, models of focal cerebral ischemia are also models of stroke. In a model of transient focal cerebral ischemia that involved intraluminal occlusion of middle cerebral artery (MCA) for around 2 h and subsequent reperfusion, MMP-9 was found to colocalize with neuronal nitric oxide synthase (nNOS), and was activated by *S*-nitrosylation.[37] Notably, nNOS activation has been implicated in the pathogenesis of ischemia as involved in producing reactive nitrogen radicals.[38] Moreover, 24 h after the reperfusion, an increase in MMP-9 activity and protein level in the ischemic hemisphere was demonstrated.[6,37] Interestingly, MMP-9 activation, as shown by *in situ* zymography, was abolished after focal ischemia in either nNOS KO mice or in wild-type mice that have been treated with nNOS-specific inhibitor.[37] There was also significant reduction in neurodegeneration in nNOS KO mice[39] and after nNOS inhibition.[37] Furthermore, incubation of neurons with S-nitrosylated (activated) MMP-9 induced their apoptosis, and this effect was abolished by treatment with MMP inhibitor.[37]

Localization studies after the transient focal cerebral ischemia showed that MMP-9 was present mainly in the endothelial cells, neutrophils, myelinated fiber tracts, and to some extent in neurons.[6,12,40,41] This localization is consistent with findings that MMP-9 in the ischemic brain degraded blood–brain barier (BBB)-associated protein, zonae occludens-1 and myelin basic protein.[6] In fact, disruption of BBB by MMP-9 was addressed repeatedly in the focal cerebral ischemia.[6,12,41,42] Furthermore, it was shown that in the model of transient focal cerebral ischemia, disruption of BBB was attenuated in MMP-9 KO mice, and that the ischemic lesions were smaller than in WT mice.[6,41] It should, however, be noted that aforementioned localization studies in ischemia were performed later than 16 h after reperfusion, when, as mentioned above, the ischemic lesions are well pronounced.[43] Moreover, neutrophils expressing MMP-9, infiltrated the cortical tissue 24 h after the reperfusion and they are the significant source of MMP-9.[42] Interestingly, the analysis of localization of MMP-9 expression made between 2 and 4 h after the reperfusion showed that MMP-9 protein and activity colocalized mainly with neurons.[7,40] These data suggest early involvement of MMP-9 in neuronal death in ischemic models, probably through degradation of laminin–neuron interactions.[7] On the other hand, Zhao *et al.*[41] showed that MMP-9 expression in neurons and astrocytes was elevated also in the later phase of stroke (7–14 days after the infarction) and was associated with neurovascular remodeling. Treatment with MMP inhibitors 7 days after the onset of ischemia suppressed neuronal plasticity, neurogenesis and angiogenesis and led to increased brain injury.[41] Late treatment with MMP inhibitor resulted also in decreasing the migration of neuroblast cells from subventricular zone into the striatum.[44] Hence, MMP-9 appears to play distinct roles in stroke pathogenesis at various times after the insult.[7,42,45–47]

In permanent focal ischemia (without reperfusion) activity of MMP-9 was reported to be elevated as early as 1 h after MCA occlusion with further increases up to 24 h, when it reached the peak activity. Concomitantly, mRNA and protein levels became also upregulated as well.[45] Interestingly, also in this model, MMP-9 KO mice were demonstrated to be more resistant to pathological lesions induced by focal cerebral ischemia in comparison with WT mice.[45] Furthermore, application of MMP inhibitors reduced the ischemic lesions.[45,46] This again suggests that MMP-9 plays an important role in the development of brain injury, but it also shows that in contrast to the global cerebral ischemia, inhibition of MMP-9 activity, either pharmacological or at the genetic level in focal cerebral ischemia, may protect brain, for example, by BBB stabilization.[6,48]

Analysis of MMP-9 expression in human stroke shows, similar to animal models, an increase in the levels of protein and gelatinolytic activity in the brain tissue after ischemic and hemorrhagic stroke.[49] MMP-9 expression within the infarct regions was mainly located around the blood vessels. In the peri-infarct areas, MMP-9 was mainly located in activated microglia and neutrophils. In addition, MMP-9 reactivity was detected also within neurons and macrophages.[49,50] It should, however, be noted that all analyzed samples were collected from patients at different age and usually relatively late after stroke, that is, 4–28 h in cases of the hemorrhagic stroke and 37–108 h in cases of the ischemic stroke.[49] Therefore, it is difficult to draw precise conclusions from this study apart from involvement of MMP-9 in human stroke.

Concluding Remarks

The extensive data reviewed above indicate that MMP-9 is a molecule of great importance for neuronal physiology and pathology. Its activation appears to be intimately linked to glutamate, acting either as a neurotransmitter, controlling long-term neuronal physiology, including synaptic plasticity, learning and memory, or acting as a potent neurotoxin. This double face of MMP-9 is hardly surprising, as it appears that this is a typical feature of a number of regulatory molecules. If active in a controlled manner, they are beneficial, however, when over-activated, they are detrimental. Hence, it appears very important to reflect on those issues when considering clinical applications of the MMP-9 inhibitors. It is conceivable that MMP inhibitors would be useful early on in the pathology of many neurological

diseases, to inhibit the detrimental aspects of MMPs, while their use later on may have adverse effects on repair. In addition, one shall also consider a possible role of MMP-9 in aberrant, epileptogenic synaptogenesis that might arise as a consequence of pathological repair.

The complex knowledge that is just being revealed about MMP-9 underscores the significance of this protein for the brain. Hence, the aforementioned studies pave the way for the next experiments aiming at the better understanding of so far underappreciated extracellular focal proteolysis and its multiple roles in the brain function and dysfunction.

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1. Woessner JF, Nagase H 2000, Oxford; New York: Oxford University Press.
2. Sternlicht MD. *Werb ZAnnu Rev Cell Dev Biol* 2001; **17**: 463–516.
3. Yong VW *et al. Nat Rev Neurosci* 2001; **2**: 502–511.
4. Olson MW *et al. J Biol Chem* 1997; **272**: 29975–29983.
5. Bruno MA, Cuello AC. *Proc Natl Acad Sci USA* 2006; **103**: 6735–6740.
6. Asahi M *et al. J Neurosci* 2001; **21**: 7724–7732.
7. Gu Z *et al. J Neurosci* 2005; **25**: 6401–6408.
8. Agrawal S *et al. J Exp Med* 2006; **203**: 1007–1019.
9. Larsen PH *et al. J Neurosci* 2003; **23**: 11127–11135.
10. Pagenstecher A *et al. J Immunol Methods* 1997; **206**: 1–9.
11. Vecil GG *et al. J Neurosci Res* 2000; **61**: 212–224.
12. Rosenberg GA *et al. Brain Res* 2001; **893**: 104–112.
13. Vaillant C *et al. J Neurosci* 1999; **19**: 4994–5004.
14. Szklarczyk A *et al. J Neurosci* 2002; **22**: 920–930.
15. Jourquin J *et al. Eur J Neurosci* 2003; **18**: 1507–1517.
16. Wilczynski GM, Kaczmarek L. *Regulation of transcription by neuronal activity* Dudde SM (ed), Springer Science.
17. Shubayev VI, Myers RR. *J Neurosci Res* 2004; **77**: 229–239.
18. Nagy V *et al. J Neurosci* 2006; **26**: 1923–1934.
19. Meighan SE *et al. J Neurochem* 2006; **96**: 1227–1241.
20. Okulski P *et al. Biol Psychiatry* 2007; doi:10.1016/j.biopsych.2006.09.012 (in press).
21. Jaworski J *et al. J Biol Chem* 1999; **274**: 28106–28112.
22. Kaczmarek L *et al. EMBO J* 2002; **21**: 6643–6648.
23. Kaczmarek L *et al. Physiol Rev* 1997; **77**: 217–255.
24. Sperk G. *Prog Neurobiol* 1994; **42**: 1–32.
25. Zagulska-Szymczak S *et al. Neurochem Int* 2001; **38**: 485–501.
26. Pollard H *et al. Neuroscience* 1994; **63**: 7–18.
27. Ben-Ari Y. *Epilepsia* 2001; **42** (Suppl 3): 5–7.
28. Zhang JW *et al. Eur J Neurosci* 1998; **10**: 3358–3368.
29. Steward O, Schuman EM. *Neuron* 2003; **40**: 347–359.
30. Tan HK *et al. Mol Cell Neurosci* 2003; **22**: 98–106.
31. Gursoy-Ozdemir Y *et al. J Clin Invest* 2004; **113**: 1447–1455.
32. Wang X *et al. J Neurosci* 2000; **20**: 7037–7042.
33. Back T *et al. J Neurol* 2004; **251**: 388–397.
34. Rivera S *et al. Eur J Neurosci* 2002; **15**: 19–32.
35. Lee SR *et al. J Neurosci* 2004; **24**: 671–678.
36. Magnoni S *et al. Neurobiol Dis* 2004; **17**: 188–197.
37. Gu Z *et al. Science* 2002; **297**: 1186–1190.
38. Won SJ *et al. J Biochem Mol Biol* 2002; **35**: 67–86.
39. Huang Z *et al. Science* 1994; **265**: 1883–1885.
40. Planas AM *et al. Neurobiol Dis* 2001; **8**: 834–846.
41. Zhao BQ *et al. Nat Med* 2006; **12**: 441–445.
42. Romanic AM *et al. Stroke* 1998; **29**: 1020–1030.
43. Garcia JH *et al. Am J Pathol* 1993; **142**: 623–635.
44. Lee SR *et al. J Neurosci* 2006; **26**: 3491–3495.
45. Asahi M *et al. J Cereb Blood Flow Metab* 2000; **20**: 1681–1689.
46. Jiang X *et al. Neurosci Lett* 2001; **305**: 41–44.
47. Machado LS, Kozak A *et al. BMC Neurosci* 2006; **7**: 56.
48. Cunningham LA *et al. Glia* 2005; **50**: 329–339.
49. Rosell A *et al. Stroke* 2006; **37**: 1399–1406.
50. Anthony DC *et al. Neuropathol Appl Neurobiol* 1997; **23**: 406–415.

Cardiac repair by stem cells

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Awake, dear heart, awake! Thou hast slept well; Awake!
William Shakespeare. The tempest. Act 1, Scene 2

Does Our Heart Last Forever?

Acknowledging the heart's regenerative capacity is important, as otherwise we would self-limit our therapeutic efforts to preserve what was left after an ischemic accident, without even thinking of other therapeutic approaches. If we measure in a mouse the fraction of cells that enter daily the mitotic cycle (determined by labelling with bromodeoxyuridine or Ki-67) and the number of dying cells (determined by labelling with apoptotic markers), we obtain the same value for both, between 0.25 and 1% of the whole cell population. This means that the whole heart must be renewed within less than 1 year.[1] In man, the rate of cardiomyocyte renewal has been estimated as 0.06% per day, so that rebuilding of the whole heart should take 4–5 years.[2] Other estimates of the cardiomyocyte cell cycle activity range between 0.0005 and 3%.[3] It is well known, however, that the cardiac parenchyma destroyed during an ischemic accident does not regenerate spontaneously. During the so-called *remodelling*, it is replaced instead by a scar of fibrous tissue, suggesting that the cardiac stem cell (CSC) potential is not enough for significant repair.

Bone Marrow Cells for Cardiac Repair

The deep interest in cardiac regeneration started in 2001 with the observation by Orlic *et al.*[4] that injected Lin^{-} $ckit^{POS}$ bone marrow cells (BMC) were able to repair acute myocardial infarcts (AMI) in mice. They did so by generating *de novo* myocardium and blood vessels, with remarkable functional recovery within 9 days. The new idea was that BMC were able to generate new cardiomyocytes, smooth muscle cells and endothelial cells. The plasticity of adult stem cells is a matter of an ardent controversy that often exceeds the scientific

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