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Title: Matrix metalloproteinase-2 and -9 as promising benefactors in development, plasticity and repair of the nervous system

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Abstract: It has been 50 years since Gross and Lapiere discovered collagenolytic activity during tadpole tail metamorphosis, which was later on revealed as MMP-1, the founding member of the matrix metalloproteinases (MMPs). Currently, MMPs constitute a large group of endoproteases that are not only able to cleave all protein components of the extracellular matrix, but also to activate or inactivate many other signaling molecules, such as receptors, adhesion molecules and growth factors. Elevated MMP levels are associated with an increasing number of injuries and disorders, such as cancer, inflammation and auto-immune diseases. Yet, MMP upregulation has also been implicated in many physiological functions such as embryonic development, wound healing and angiogenesis and therefore, these proteinases are considered to be crucial mediators in many biological processes. Over the past decennia, MMP research has gained considerable attention in several pathologies, most prominently in the field of cancer metastasis, and more recent investigations also focus on the nervous system, with a striking emphasis on the gelatinases, MMP-2 and MMP-9. Unfortunately, the contribution of these gelatinases to neuropathological disorders, like multiple sclerosis and Alzheimer's disease, has overshadowed their potential as modulators of fundamental nervous system functions. Within this review, we wish to highlight the currently known or suggested actions of MMP-2 and MMP-9 in the developing and adult nervous system and their potential to improve repair or regeneration after nervous system injury.

Leuven, March 1, 2012

Prof. Michael J. Zigmond
Editor-in-Chief, Progress in Neurobiology
Mrs. Susan D. Giegel
Managing editor

Dear Prof. Zigmond,
Dear Mrs. Giegel,

We are pleased to perceive that our manuscript 'Matrix metalloproteinase-2 and -9 as promising benefactors in development, plasticity and repair of the nervous system' (PRONEU-D-12-00097) is accepted for publication.

We wish to thank the members of the Editorial Board and the reviewers for their thorough analysis and valuable comments. All changes related to the reviewers' remarks are mentioned point-by-point in the response letter. As suggested, we included a more detailed table and two extra figures, depicting gelatinase functions in CNS development and adult plasticity.

We hope that our revised manuscript is now suitable for publication in 'Progress in Neurobiology'.

Sincerely,

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Highlights

- In the nervous system, MMP-2 and -9 actions go far beyond their detrimental roles
- MMP-2 and -9 are important in many developmental events in the nervous system
- MMP-9 is a key regulator of synaptic plasticity in the healthy adult brain
- MMP-2 and -9 promote regeneration and repair of the injured nervous system

Matrix metalloproteinase-2 and -9 as promising benefactors in development, plasticity and repair of the nervous system

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Running title: Beneficial role of MMP-2 and MMP-9 in the CNS

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ABSTRACT

It has been 50 years since Gross and Lapiere discovered collagenolytic activity during tadpole tail metamorphosis, which was later on revealed as MMP-1, the founding member of the matrix metalloproteinases (MMPs). Currently, MMPs constitute a large group of endoproteases that are not only able to cleave all protein components of the extracellular matrix, but also to activate or inactivate many other signaling molecules, such as receptors, adhesion molecules and growth factors. Elevated MMP levels are associated with an increasing number of injuries and disorders, such as cancer, inflammation and auto-immune diseases. Yet, MMP upregulation has also been implicated in many physiological functions such as embryonic development, wound healing and angiogenesis and therefore, these proteinases are considered to be crucial mediators in many biological processes.

Over the past decennia, MMP research has gained considerable attention in several pathologies, most prominently in the field of cancer metastasis, and more recent investigations also focus on the nervous system, with a striking emphasis on the gelatinases, MMP-2 and MMP-9. Unfortunately, the contribution of these gelatinases to neuropathological disorders, like multiple sclerosis and Alzheimer's disease, has overshadowed their potential as modulators of fundamental nervous system functions. Within this review, we wish to highlight the currently known or suggested actions of MMP-2 and MMP-9 in the developing and adult nervous system and their potential to improve repair or regeneration after nervous system injury.

1. INTRODUCTION

1.1 THE MATRIX METALLOPROTEINASE FAMILY

Matrix metalloproteinases (MMPs) constitute a family of over 20 Zn^{2+} -dependent proteolytic enzymes that are able to cleave all structural components in the extracellular environment and many biologically active molecules involved in signal transduction. All MMPs share a basic structural organisation, but based on their substrate specificity and domain organization, they can be further arranged into several subgroups: the collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs (MT-MMPs) and ‘others’ (for an overview see (Nagase *et al.*, 2006). Many different stimuli, such as growth factors, cytokines, reactive oxygen species etc., can trigger MMP expression in physiological as well as pathological conditions (Nagase *et al.*, 2006; Sternlicht and Werb, 2001). Following their production as inactive zymogens (pro-MMPs), disruption of the intramolecular complex between the single cysteine residue in its propeptide domain and the essential zinc atom in the catalytic domain, is required for activation (Van Wart and Birkedal-Hansen, 1990). MMP activity is restricted by reversion-inducing cysteine-rich protein with kazal motifs (RECK) and $\alpha 2$ -macroglobulin, and predominantly by tissue inhibitors of metalloproteinases (TIMPs). All 4 currently identified TIMPs bind MMPs non-covalently in a 1:1 stoichiometric ratio and with variable affinity. Besides these endogenous inhibitors, many other mechanisms, like cell compartmentalisation and post-translational control, regulate MMP activity (Chakraborti *et al.*, 2003). Their broad spectrum of substrates and their expression by different cell types and tissues all over the body implicate the involvement of these proteinases in a wide range of physiological processes. Indeed, MMPs are known to be involved in cell death, proliferation, differentiation, migration and cell signaling and play important roles during tissue remodeling, thereby contributing to e.g. angiogenesis, organogenesis and wound healing. On the contrary, uncontrolled MMP activities or an impaired MMP/TIMP balance can amplify or induce

various diseases/disorders, such as tumor metastasis and atherosclerosis. Also in the CNS, upregulation of MMPs is known to aggravate many neurological disorders like stroke, multiple sclerosis (MS), Parkinson's (PD) and Alzheimer's disease (AD) (Sternlicht and Werb, 2001; Yong *et al.*, 2007). Although an aberrant MMP activity in CNS pathologies has been extensively described, evidence pointing towards the participation of MMPs in CNS development, recovery and regeneration is also emerging. Apparently, the biological activities of MMPs seem to be much more complex than initially thought.

1.2 THE GELATINASE SUBFAMILY

Until now, the gelatinases are the most extensively studied subfamily of MMPs. MMP-2 (gelatinase A, 72 kDa type IV collagenase) and MMP-9 (gelatinase B, 92 kDa type IV collagenase) contain 3 fibronectin type II repeats in their catalytic site that facilitate binding of denatured type IV and V collagen (=gelatine), elastin and collagen (Steffensen *et al.*, 1995). Pro-MMP-2 is constitutively expressed and predominantly activated via binding to a TIMP-2/MT1-MMP complex on the cell surface, where TIMP-2 serves as a receptor for MMP-2. A TIMP-2-free neighbouring MT1-MMP cleaves the bound MMP-2 propeptide and autolytic mechanisms subsequently generate the fully active MMP-2, which is then released in the pericellular space (Itoh *et al.*, 2001; Strongin *et al.*, 1995). While only low levels of TIMP-2 will allow MMP-2 activation at the cell surface within this ternary complex, high TIMP-2 concentrations inhibit net MMP-2 proteolytic activity in the pericellular space (Bernardo and Fridman, 2003). Also activated protein C, MT2-MMP and thrombin are known MMP-2 activators (Galis *et al.*, 1997; Morrison *et al.*, 2001; Nguyen *et al.*, 2000). On the other hand, pro-MMP-9 can be activated by cleavage of the prodomain through proteolytic enzymes, such as MMP-3 and plasmin, or by disruption of the cysteine switch by e.g. NO, S-nitrosylation or organomercurials under pathophysiological or stress conditions (Gu *et al.*,

2002; Ogata *et al.*, 1992; Ridnour *et al.*, 2007).

For both gelatinases, and especially for MMP-9, a role in several physiological processes, including wound healing, blastocyst implantation and bone growth, has been reported. However, both MMPs seem importantly involved in many more pathological conditions, such as infectious and inflammatory diseases, cancer and cardiovascular disorders (Klein and Bischoff, 2011; Stamenkovic, 2003; Van den Steen *et al.*, 2002).

1.3 A DETRIMENTAL ROLE FOR MMP-2 AND MMP-9 IN THE NERVOUS SYSTEM

The first evidence for a contribution of MMPs to brain injury and neuroinflammation has been assigned to the gelatinase subfamily, which is shown to increase the blood-brain barrier (BBB) permeability by degrading the vascular basal lamina and/or tight junctions between cells within the neurovascular unit (Rosenberg, 1995, 2002, 2009; Rosenberg *et al.*, 1998; Rosenberg *et al.*, 1992; Yang *et al.*, 2007). This is particularly the case for MMP-9, which is found to be highly increased in e.g. microglial cells in response to inflammatory events (Giraudon *et al.*, 1996; Gottschall *et al.*, 1995). Next to BBB disruption, MMP-9 can induce neuronal cell death, as observed during excitotoxicity and global cerebral ischemia. As such, hippocampal neurons are protected from kainate-induced excitotoxicity after administration of a MMP-9 inhibitor (S24994) (which has been chemically modified to enhance MMP-9 inhibition) and MMP-9 deficient mice show significantly reduced neuronal damage in the hippocampus after transient global cerebral ischemia (Jourquin *et al.*, 2003; Lee *et al.*, 2004). Both gelatinases are also involved in myelin breakdown and facilitate immune cell extravasation after BBB disruption, thereby contributing to MS (Chandler *et al.*, 1995). More specifically, increased MMP-2 and MMP-9 levels are demonstrated in the cerebrospinal fluid (CSF) and/or serum of MS patients, and both gelatinases are detected in and around MS plaques (Avolio *et al.*, 2003; Gijbels *et al.*, 1992; Leppert *et al.*, 1998). Also,

the acute MMP-9 upregulation after spinal cord injury might promote blood-spinal cord barrier disruption, neutrophil invasion and secondary myelin degradation (Noble *et al.*, 2002). Using gelatin zymography, a rapid and transient upregulation of MMP-9 and a delayed and persistent upregulation of MMP-2 is demonstrated in dorsal root ganglia after spinal nerve ligation in rat. Different approaches in mice consequently show that the development of nerve injury-induced neuropathic pain relies on MMP-9 and MMP-2, respectively raised during the early and late phase (Kawasaki *et al.*, 2008). An increase in MMP-2 activity is also found in serum of patients suffering from migraine with aura whereas migraine patients without aura show elevated MMP-9 levels (Martins-Oliveira *et al.*, 2009). In addition, MMP-9 upregulation is implicated in the pathogenesis of epilepsy (Wilczynski *et al.*, 2008). Infectious CNS diseases such as meningitis and viral encephalitis are also correlated with elevated MMP-2/-9 levels, (Giraudon *et al.*, 1996; Kanoh *et al.*, 2008; Leppert *et al.*, 2001; Martinez-Torres *et al.*, 2004). In the CSF of patients with vascular dementia (VaD), high concentrations of MMP-9 are detected, while MMP-2 levels remain unaltered (Adair *et al.*, 2004). In AD patients, an increase in MMP-9 expression is detected in neurons, neurofibrillary tangles, senile plaques and plasma but not in CSF, while also here, MMP-2 levels are unchanged in CSF, plasma or brain (Adair *et al.*, 2004; Asahina *et al.*, 2001; Backstrom *et al.*, 1996; Backstrom *et al.*, 1992; Lorenzl *et al.*, 2003). Serum levels of both gelatinases are also correlated with amyotrophic lateral sclerosis (Niebroj-Dobosz *et al.*, 2010; Sokolowska *et al.*, 2009). In glioblastoma cells, both MMP-2 and MMP-9 expression and activity levels are clearly elevated, thereby interacting with ECM components and adhesion molecules and supporting cancer progression, neovascularization and metastasis in the CNS (Forsyth *et al.*, 1999; Lampert *et al.*, 1998).

Thus, although only briefly summarized above, ample evidence exists and associates gelatinases with pathophysiological processes in the nervous system, including barrier

disruption, neuroinflammation, demyelination, brain edema, etc. Nevertheless, the biological significance of these MMPs is much more complex and certainly not limited to their detrimental role. Novel recently identified substrates, along with spatiotemporal expression patterns and functional evidence, support a beneficial contribution of both gelatinases to development, repair and regeneration of the nervous system.

2. FUNCTIONAL IMPLICATIONS FOR MMP-2 AND MMP-9 IN THE DEVELOPING NERVOUS SYSTEM

MMPs are highly expressed in the developmental nervous system. More specifically for MMP-2 and MMP-9, a high mRNA expression is detected in the murine CNS during the first postnatal week, whereafter MMP-2 mRNA levels globally drop. MMP-9 mRNA decreases in the brain but not in the spinal cord, which shows a transient increase in MMP-9 mRNA levels in five weeks old mice (Ayoub *et al.*, 2005; Bednarek *et al.*, 2009; Ulrich *et al.*, 2005). MMP-2 protein is mainly expressed in astrocytes, but also found in some cortical and cerebellar neurons and in endothelial cells and pericytes (Ayoub *et al.*, 2005; Planas *et al.*, 2001; Szklarczyk *et al.*, 2002). In contrast, MMP-9 expression in the CNS is found to be more restricted to neurons and myelinated fiber tracts in the hippocampus, corpus callosum, striatum and cortex (Planas *et al.*, 2001; Szklarczyk *et al.*, 2002; Zhang *et al.*, 1998). As MMPs are important modifiers of the cellular and extracellular environment, these expression data clearly suggest an intimate relationship between the gelatinases and remodeling events necessary during CNS development (Rivera *et al.*, 2010). Their contribution in these different developmental processes are described below and represented in Table 1 and Fig. 1.

2.1 The gelatinases as possible regulators of developmental vascularisation

MMP-2 and MMP-9 are importantly involved in angiogenic processes during tumor

formation (Bergers *et al.*, 2000; Fang *et al.*, 2000; Huang *et al.*, 2002; Itoh *et al.*, 1998), but both gelatinases also seem to participate in developmental vascularization. The observed MMP-9 mRNA expression in progenitor and/or differentiated neural cells, which are closely associated with the PECAM-1 positive developing vascular endothelium in murine embryos, might indicate that MMP-9 contributes to blood vessel development, however evidence supporting this hypothesis remains very poor (Canete Soler *et al.*, 1995). In the developing human telencephalon, MMP-2 expression, which is observed predominantly by pericytes, is shown to correlate with the initial phase of vessel sprouting by cleavage of collagen IV in the basement membrane, thereby contributing to blood-brain barrier differentiation and organization of the growing vessel wall (Fig. 1A) (Girolamo *et al.*, 2004; Virgintino *et al.*, 2007).

Together, these findings seemingly indicate a prominent contribution for MMP-2 and MMP-9 in the interplay between early angiogenesis and a correct CNS development.

2.2 MMP-2 and MMP-9 are involved in developmental neurogenesis

The first report describing the presence of MMPs in neuroectodermal precursor cells show MMP-2, but not MMP-9 expression in human embryonic CNS stem cells (Frolichsthal-Schoeller *et al.*, 1999). In chicken, MMP-2 seems essential in neurogenesis. During early embryogenesis, chicken neural crest cells (NCCs) detach from the neural epithelium and undergo an epithelial-mesenchymal transition. The initial MMP-2 expression by the NCCs, seen upon their detachment from the neural epithelium, rapidly decreases as these mesenchymal cells disperse. Importantly, further *in vivo* and *in vitro* experiments show a disturbed migration-induced mesenchymal transition of NCCs in the avian embryo after MMP-2 inhibition via antisense morpholino oligonucleotides (Fig. 1B) (Duong and Erickson, 2004).

Recently, also MMP-9 is found to be implicated in neural stem cell (NSC) proliferation in the embryonic brain (Fig. 1B). Culturing NSCs, isolated from the embryonic rat cortex, under lowered O₂ pressure, thereby mimicking the *in vivo* neurogenic stem cell niche, leads to increased MMP-9 mRNA and protein levels and an increased NSC proliferation and subsequent migration. Treatment with a synthetic MMP inhibitor with the highest affinity to MMP-9 results in reduced NSC proliferation, presumably via modulation of the Wnt/ β -catenin signaling pathway, and decreases NSC migration (Ingraham *et al.*, 2011; Wu *et al.*, 2007). These findings are in accordance with the reported MMP-9 mRNA expression in neurogenic niches of mouse embryos (Canete Soler *et al.*, 1995). Here, mitotically active NSCs differentiate into specific neuronal cell types, a process controlled by intracellular and extracellular processes including cytoskeletal modifications, reorganization of the ECM and cell-cell interactions, which can all possibly be influenced by gelatinases (Wojcik-Stanaszek *et al.*). Furthermore, also in *in vitro* differentiation of neuroblastoma cells, which resemble neural crest-derived cells, MMP-9 upregulation correlates with the transition of neuroblastoma cells to GAP-43 immunopositive neuron-like cells (Higgins *et al.*, 2009).

These results suggest that MMP-9 and MMP-2 might be potent regulators of NSC proliferation, migration and differentiation, however, thus far only reported by a limited number of studies. Importantly and compared to NSC neurogenesis, MMP-9 seems not involved in postnatal neuronal proliferation. Indeed, no difference is detected in the number of proliferating granule cells (GCs) in the developing postnatal cerebellum of MMP-9 deficient mice, as compared to wild-type animals (Vaillant *et al.*, 2003). Nonetheless, recent evidence suggest an important role for MMP-2 during early postnatal GC proliferation in the mouse cerebellar cortex, thereby influencing cell cycle kinetics, leading to a prolonged S-phase duration (Verslegers *et al.*, 2013, unpublished data).

Nonetheless, further studies investigating neurogenesis and neuronal proliferation are

clearly needed to shed more light onto the involvement of MMP-2 and MMP-9 in these developmental processes.

2.3 A contribution of MMP-2 and MMP-9 to neuron and astrocyte migration

MMPs are often suggested to influence cell migration in the developing nervous system (Fig. 1C) (Amberger *et al.*, 1997; Del Bigio *et al.*, 1999; Giambernardi *et al.*, 2001). Based on their protein distribution, both MMP-2 and MMP-9 are believed to be implicated in neuronal migration during postnatal cerebellar development (Ayoub *et al.*, 2005; Vaillant *et al.*, 1999). A delayed GC radial migration is observed in MMP-9 deficient pups and in *ex vivo* cerebellar slices incubated with a MMP-2/MMP-9 inhibitor. However, this disturbed migration might be secondary to an impaired GC neurite outgrowth, demonstrated in cerebellar explant cultures incubated with a MMP-9 blocking antibody, and resulting in stalling of GCs in the external granular layer (Ayoub *et al.*, 2005; Vaillant *et al.*, 2003).

Furthermore, also astrocyte migration is thought to be associated with gelatinase activity. MMP-2 and MMP-9 mRNA are observed in cultured astrocytes obtained from neonatal mouse brain. Immunostainings and gel zymography reveal only limited levels of MMP-9, while MMP-2 seems to be constitutively expressed in astrocytes and shows a 10-fold higher activity compared to MMP-9 in astrocyte supernatant. Moreover, administration of a selective MMP-9 inhibitor ($IC_{50}(\text{MMP-9})=0.0025\text{nM}$, $IC_{50}(\text{MMP-2})=0.7\text{nM}$) does not affect astrocyte migration, while administering an inhibitor more selectively for MMP-2 ($IC_{50}(\text{MMP-2})=0.06\text{nM}$, $IC_{50}(\text{MMP-9})=0.5\text{nM}$) reduces it by 35%, presumably via an interplay between $\beta 1$ -integrin and the cytoskeleton (Ogier *et al.*, 2006). Indeed, MMP-2 co-localizes with $\beta 1$ -integrin on the cell membrane and may influence integrin-dependent cell motility pathways through cleavage of the $\beta 1$ -integrin ectodomain (Fig. 1C) (Kryczka *et al.*, 2012; Moissoglu and Schwartz, 2006; Ogier *et al.*, 2006).

Besides their extracellular actions, MMPs are also observed intracellularly, where they can be activated by e.g. phosphorylation processes or splice variants lacking a signal sequence. Besides, they can be taken up by the cell via clathrin-dependent or independent endocytosis mechanisms after extracellular activation (for more examples of intracellular activation and transport mechanisms, we refer to a recently published extensive review by Cauwe and Opdenakker (Cauwe and Opdenakker, 2010). An important fraction of the intracellular gelatinase substrates are cytoskeletal components (Fig. 1C) (Cauwe and Opdenakker, 2010; Schram *et al.*, 2011). Therefore, by promoting neurite outgrowth and/or astrocyte process elongation, MMPs might also contribute to neuronal/astrocytic migration.

Overall, MMP-2 and MMP-9 seem to support migration in the developing nervous system, similar to what is reported for several non-neuronal cell populations (Murphy and Gavrilovic, 1999). Yet, additional future *in vivo* experiments are needed to amplify the evidence relating gelatinase activity to neuron and astrocyte migration.

2.4 MMP-2 and MMP-9 regulate dendritic and axonal outgrowth and guidance

During mouse embryogenesis, more specifically between 7,5 to 15 days of gestation, MMP-9 mRNA levels are not only high in germinal zone cells, as mentioned above, but are also detectable in differentiating cortical cells. Therefore, a role for MMP-9 in supporting neurite extension of differentiating cells can be suggested (Canete Soler *et al.*, 1995). In addition, gelatinolytic activity is demonstrated in the entire neocortex of the embryonic mouse (Gonthier *et al.*, 2009). MMP-2 is highly expressed in apical dendrites of cortical neurons, and its expression and activity can be mediated by the dendritic growth-promoting molecule Sema3A, suggesting an involvement of this gelatinase in the Sema3A growth-promoting pathway (Fig. 1D) (Gonthier *et al.*, 2009). Also electrical stimulation of sympathetic neurons, isolated from rat superior cervical ganglia, induces MMP-2 secretion and activation, and

augments neurite outgrowth. This MMP-2-mediated nerve sprouting relies on the conversion of pro-nerve growth factor (NGF) into mature NGF, which subsequently binds the tyrosine kinase A (TrkA) receptor (Fig. 1D) (Saygili *et al.*, 2011). Furthermore, both gelatinases show marked upregulated gene and protein levels in cultured murine neuroblastoma cells transfected with choline acetyltransferase, inducing the differentiation of cells to a neuron-like phenotype and associated with extensive neurite extension (Anelli *et al.*, 2007). MMP-2 and MMP-9 are also able to cleave the intercellular adhesion molecule ICAM-5 and might as such support dendritic outgrowth and spine remodelling (Fig. 1D) (Tian *et al.*, 2007). Indeed, ICAM-5 has been described to form homophilic interactions leading to dendritic outgrowth (Tamada *et al.*, 1998; Tian *et al.*, 2000), whereas the cytoplasmic domain interacts with ERM (erzin/radixin/moesin) family proteins which initiate the formation of filopodia via the actin cytoskeleton (Fig. 1D) (Furutani *et al.*, 2007). Additionally, MMP-9 is also known to contribute to neurite outgrowth of GCs, as shown in cerebellar explant cultures, but here the underlying working mechanism remains elusive (Vaillant *et al.*, 2003). Importantly, MMP-9 is postulated to influence axonal growth and guidance through neural cell adhesion molecule (NCAM) signaling. Posttranslational modification of NCAM is necessary to induce proper neurite outgrowth and guidance (Niethammer *et al.*, 2002), and these modifications are induced by FGF-2 (Ponimaskin *et al.*, 2008). As FGF-2 can influence MMP-9 expression (Higgins *et al.*, 2009), which is in turn known to modify NCAM (Fujita-Hamabe and Tokuyama, 2012; Shichi *et al.*, 2011), one can easily imagine a potential role for MMP-9 during NCAM-mediated axonal growth and guidance (Fig. 1D).

Indeed, MMP-9 is also described to influence axon guidance. Axonal growth cones are responsible for interpreting adhesive and repulsive cues of the ECM and guide axons en route to their appropriate targets. Among these guiding signals, ephrins and their respective receptors are known to be regulated by MMPs. As such, the EphB2 receptor is cleaved by

MMP-9 after interaction with its ligand ephrin-B2, thereby inducing cell-cell repulsion through cytoskeletal responses, as demonstrated in cultured hippocampal neurons and confirmed via Western blotting experiments on embryonic mouse brain extracts (Fig. 1D) (Lin *et al.*, 2008). Furthermore, MMP-2 seems important during retinal ganglion cell (RGC) axonal guidance. Although MMP-9 mRNA is detected in RGCs in the murine eye, it does not seem to be present in *Xenopus* eyes (Canete Soler *et al.*, 1995; Carinato *et al.*, 2000). The aberrant RGC axonal guidance towards the optic tectum, which is observed after addition of GM6001, a broad-spectrum MMP inhibitor, and SB-3CT, a gelatinase specific inhibitor, in *Xenopus* exposed brain preparations, is therefore attributed to MMP-2 (Hehr *et al.*, 2005). Indeed, MMP-2 expression is observed along the optic tract and around the tectum in *Xenopus*, where it is suggested to influence RGC axonal guidance, for instance by ectodomain shedding of fibroblast growth factor receptor 1 (FGFR1) on RGC growth cones (Hehr *et al.*, 2005; Levi *et al.*, 1996; McFarlane *et al.*, 1995). Of note, MMP-2 expression is also reported in normal human adult RGCs and their axons (Agapova *et al.*, 2001; Hehr *et al.*, 2005), and in growth cones of dorsal root ganglionic neurons from cultured chick embryos and neonatal rats (Tominaga *et al.*, 2009; Zuo *et al.*, 1998).

From all these studies it can be concluded that MMP-2 and MMP-9 influence dendritic and axonal outgrowth and guidance in the developing CNS, and thereby contribute to an overall correct neuronal wiring.

2.5 MMP-2 and MMP-9 are involved in oligodendrocyte differentiation and myelination

When oligodendrocytes, the myelinating cells of the CNS, extend their multiple branched processes towards growing axons, remodeling of the ECM and release of signalling molecules is required, and supposed to rely at least in part, on MMP activity. In the rodent CNS, MMP-9 is located in specific areas associated with developmental myelination

processes, such as in the spinal cord at sites where oligodendrocyte precursors reside, and in the corpus callosum, a site of oligodendrocyte process outgrowth (Uhm *et al.*, 1998; Ulrich *et al.*, 2005). In the corpus callosum, upregulated MMP-9 expression coincides with a temporal increase in myelination, while MMP-9 deficient mice show a highly reduced myelination in this area, thereby supporting a role for MMP-9 in influencing postnatal CNS oligodendrocyte maturation. Moreover, MMP-9 expression might be regulated upstream by protein kinase C (PKC), as cultured human oligodendrocytes show an increase in MMP-9 activity after administration of pharmacological PKC activators, leading to an increase in process budding and extension during the initial phase of myelination (Larsen *et al.*, 2006; Uhm *et al.*, 1998). Also in the mouse optic nerve, MMP-9 expression and activity increases during myelin formation and is located at the growing tips of the oligodendrocytes, suggestive for an important role of MMP-9 during the initial phase of myelination (Fig. 1E) (Oh *et al.*, 1999).

Recently, also an involvement for MMP-2 in myelination is described, although only in the peripheral nervous system (PNS), during the early phase of myelination (Fig. 1E). MMP-2 is expressed by Schwann cells (SCs) and its early expression and activity correlates with the degree of myelination in dorsal root neurons co-cultured with SCs. Moreover, myelin formation is incomplete after blocking MMP-2 with either EDTA, a broad-spectrum MMP inhibitor, or an inhibitor specifically designed to target MMP-2/-9 ($C_{21}H_{19}NO_4S$) (Lehmann *et al.*, 2009). Furthermore, both MMP-2 and MMP-9 are recently associated with sciatic nerve myelination *in vivo*, more specifically through a correct processing of β -dystroglycan in the SC cytoplasm. These β -dystroglycan complexes are needed to assure a proper segregation of functional proteins into specific cellular subdomains, which is necessary for SC signalling, metabolic and transport functions during developmental myelination (Court *et al.*, 2011).

In general, in the CNS, MMP-9 seems an important mediator of oligodendrocyte maturation and myelination, mainly during the initial phase of myelination. Additional studies

are required, as up to now the gelatinases are only shown to be functionally relevant in limited brain areas and only in the PNS in the case of MMP-2.

2.6 Are the gelatinases potent regulators of synaptic remodeling?

Studies focussing on the role of MMPs in functional synaptogenesis during nervous system development are largely lacking, in comparison to those investigating learning-dependent plasticity and reactive synaptogenesis following injury (Ethell and Ethell, 2007). As developmental plasticity processes resemble those during adult plasticity and repair mechanisms, and as MMP-9 (and MMP-2) is an important and widely known regulator of learning-dependent and post-injury reactive synaptogenesis (summarized in section 3.1; see also Table 1), it can be postulated that gelatinases might be general mediators of synaptogenesis during CNS development.

3. FUNCTIONAL IMPLICATIONS FOR MMP-2 AND MMP-9 IN PLASTICITY PROCESSES IN THE ADULT HEALTHY BRAIN

3.1 A convincing role for MMP-9 in hippocampal-dependent plasticity

Long-term potentiation (LTP), used to study synaptic plasticity, is defined as an increase in synaptic strength underlying learning and memory and is characterized by long-term structural remodeling of several ECM and cell-surface molecules, leading to the necessary dendritic modifications, as well as by delivery and activation of NMDA receptors (NMDARs) and AMPARs at the synaptic cleft. Although the mechanisms controlling these processes remain poorly understood, recent studies point towards the importance of MMPs in regulating the required extracellular proteolysis and neurotransmitter receptor trafficking and activation (Bozdagi *et al.*, 2007; Michaluk *et al.*, 2009). Of all MMPs, especially MMP-9 is repeatedly investigated and seems to be a very potent regulator of hippocampal LTP (see also

Table 1 and Fig. 2), more specifically in the dentate gyrus, in Schaffer collateral projections on CA1 dendrites, and in the mossy fiber-CA3 projections (Bozdagi *et al.*, 2007; Gawlak *et al.*, 2009; Konopacki *et al.*, 2007; Nagy *et al.*, 2006; Szklarczyk *et al.*, 2002; Wang *et al.*, 2008; Wiera *et al.*, 2012). Notably, administration of pharmacological inhibitors (MMP-2/-9 inhibitor II: IC₅₀(MMP-2)=17nM, IC₅₀(MMP-9)=30nM), or function-blocking antibodies to MMP-9 *in vivo* and in hippocampal slice cultures, revealed that this protease serves as a modulator of LTP maintenance but not LTP induction or long-term depression (Bozdagi *et al.*, 2007; Nagy *et al.*, 2006). During LTP, MMP-9 mRNA is transported along microtubules towards spines, where it is translated and activated after LTP-inducing stimuli (Fig. 2A-C) (Dziembowska *et al.*, 2012; Sbaji *et al.*, 2008). Furthermore, mice subjected to a Morris water maze test (MWM) display a transient increase in hippocampal MMP-9 levels while MMP-9 deficient animals show behavioral impairments and memory deficits after hippocampus-dependent associative learning (Meighan *et al.*, 2006; Nagy *et al.*, 2006). Also transgenic mice overexpressing tPA, an upstream regulator of MMP-9, display shorter learning acquisition times in the MWM (Hu *et al.*, 2006; Madani *et al.*, 1999), again suggestive for a role of MMP-9 in hippocampal LTP.

LTP is mainly influenced by changes in post-synaptic Ca²⁺ currents which are regulated by activation of L-type voltage-dependent Ca²⁺ channels (LVDCCs) (Huber *et al.*, 1995; Kapur *et al.*, 1998; Niikura *et al.*, 2004), or NMDARs (Grosshans *et al.*, 2002). Active MMP-9 might influence the activation of LVDCCs, possibly by cleaving ECM molecules that can form functional complexes with these receptors, such as tenascin-C (Fig. 2D) (Dityatev and Schachner, 2003; Evers *et al.*, 2002; Strekalova *et al.*, 2002), which is a well known MMP-9 substrate (Kalembeiyi *et al.*, 2003). Additionally, in rat hippocampal excitatory synapses, gelatinolytic activity is associated with NMDARs and AMPARs after kainate treatment (Gawlak *et al.*, 2009). MMP-9 may influence hippocampal NMDAR-dependent

plasticity by inducing NMDAR surface trafficking between non-synaptic and synaptic compartments, that largely relies on integrin signaling. As such, matrix molecules released after MMP-9 mediated processing might possibly act as ligands for these integrins, which are in turn responsible for NMDAR trafficking and subsequent activation via phosphorylation (Fig. 2, E) (Chen and Roche, 2007). Indeed, blocking the integrin $\beta 1$ subunit in the presence of active MMP-9, results in complete abolishment of NMDAR mobility (Michaluk *et al.*, 2009). NMDAR trafficking and subsequent activation is also believed to be affected by EphB receptors and β -dystroglycan, molecules known as MMP-9 substrates and able to interact with glutamate receptors, either directly or indirectly (Fig. 2F) (Lin *et al.*, 2008; Michaluk *et al.*, 2007; Takasu *et al.*, 2002; Wyszynski *et al.*, 1997). Indeed, MMP-9 and β -dystroglycan both co-localize at postsynaptic spines during enhanced neuronal activity, induced by pharmacological seizure induction (Michaluk *et al.*, 2007). Notably, MMP-9 dependent NMDAR activation is reported to lead to a subsequent increase in MMP-9 protein levels, thereby further promoting LTP (Nagy *et al.*, 2006).

Besides NMDAR/LVDCC activation, spine modifications such as enlargement of dendritic spines, form a prerequisite for maintaining and stabilizing LTP, as they reflect differential strengthening of synaptic connections in response to experience (Holtmaat *et al.*, 2006). Herein, a role for MMP-9 has been assigned (see Fig. 2). An LTP-induced ICAM-5 cleavage by MMP-9 in cultured hippocampal neurons was found to result in elevated firing rates accompanied by an increase in the amount and size of spine heads and in elongation of dendritic filopodia. These processes are believed to result from binding of the soluble ICAM-5 ectodomain to $\beta 1$ -integrins (Fig. 2, G) (Conant *et al.*, 2011; Niedringhaus *et al.*, 2012; Tian *et al.*, 2007), and are suppressed when subjecting hippocampal neurons to gelatinase inhibitors (MMP-2/-9 inhibitor II and MMP-9 inhibitor I ($IC_{50}=5nM$)) (Tian *et al.*, 2007). Spine modifications are particularly modulated by the actin cytoskeleton and by postsynaptic

transmembrane proteins, among which several known MMP substrates are already identified (Lin and Koleske, 2010). Moreover, LTP correlates with a clear punctate MMP-9 distribution and activity observed within hippocampal excitatory dendritic spines (Sbai *et al.*, 2008). The importance of MMP-9 in dendritic morphological alterations is further investigated in transgenic rats overexpressing auto-activating MMP-9 (Michaluk *et al.*, 2011; Wilczynski *et al.*, 2008) and in organotypic hippocampal slice cultures incubated with recombinant MMP-9 (rMMP-9) (Wang *et al.*, 2008) or auto-activating rMMP-9 (Michaluk *et al.*, 2011). These studies reveal a clear involvement of MMP-9 in thinning and elongation of dendritic spines as well as enlargement of spine heads. Notably, incubation of cultured hippocampal neurons with inactive rMMP-9 results in the abolishment of spine modifications and synaptic potentiation (Michaluk *et al.*, 2011). Similar as for NMDAR activation and diffusion, these remodeling events seem to be mediated by integrin signaling resulting in actin polymerization and stabilization. Indeed, the MMP-9-mediated induction of synaptic strength is attenuated after addition of integrin function-blocking reagents (Kim *et al.*, 2009; Kramar *et al.*, 2006; Nagy *et al.*, 2006). The effect of MMP-9 on integrins is suggested to result in cleavage of laminin or other ECM molecules, thereby making Arg-Gly-Asp (RGD) sites on these proteins accessible for cells to attach, and recognizable for integrins present on the cell surface (Fig. 2H) (Ethell and Ethell, 2007; Ruoslahti, 1996). Other possible underlying mechanisms via which MMP-9 affects LTP-dependent spine remodeling were discovered via a 2D-DIGE (two-dimensional difference in gel electrophoresis) experiment, revealing collapsin response mediator protein 2 (CRMP-2) and synaptic cell adhesion molecule 2 (SynCAM-2) as downstream targets of MMP-9 (Bajor *et al.*, 2012). CRMP-2 influences rearrangement of the cytoskeleton via microtubule assembly at the postsynaptic site (Fig. 2, I) (Arimura *et al.*, 2005; Fukata *et al.*, 2002), thereby possibly mediating dendritic patterning and spine outgrowth. Presynaptically, CRMP-2 is involved in stimulating glutamate release via increased Ca^{2+}

uptake through Cav2.2 channels (Fig. 2J) (Brittain *et al.*, 2009). SynCAM-2 might be involved in cell-cell association and synaptic plasticity at the synapse (Fig. 2K) (Biederer *et al.*, 2002; Fogel *et al.*, 2007; Sara *et al.*, 2005). Furthermore, MMP-9 is also shown to induce the receptor-mediated α -secretase-like cleavage of amyloid precursor protein (APP), at least *in vitro*, via NGF activation, leading to secretion of sAPP α , known to contribute to neuronal plasticity and memory formation (Fragkouli *et al.*, 2011). MMP-9-dependent APP cleavage can be linked to hippocampal LTP, as elevated sAPP α levels are observed in the hippocampus and cortex of transgenic mice over-expressing MMP-9. Moreover, these transgenic mice also show increased dendritic spine density, enhanced cognitive performance and prolonged maintenance of LTP in hippocampal slices (Fragkouli *et al.*, 2012).

Overall, these results illustrate a well-documented role for MMP-9 as a major modulator of hippocampal plasticity, more specifically its involvement in the maintenance of LTP via its effect on NMDAR activation/trafficking and alterations in spine morphology.

3.2 Does MMP-2 play a role in hippocampal-dependent plasticity?

In contradiction to MMP-9, MMP-2 seems not involved in long-term hippocampal plasticity processes. MMP-2 activity does not increase after LTP induction, as shown by gelatin zymography and Western blotting on LTP-induced and control tissue extracts (Nagy *et al.*, 2006; Wiera *et al.*, 2012). However, a possible role for this gelatinase in hippocampal plasticity should not be denied. For this matter, it is worth mentioning that hippocampal plasticity does not only depend on late LTP but instead is preceded by an initial LTP induction (early LTP), regulated at the presynaptic domain, followed by a short term potentiation at the postsynaptic site, both taking only a few seconds to hours (Huang, 1998; Volianskis and Jensen, 2003). Importantly, induction of early and late LTP in hippocampal slices requires two distinct stimulus protocols (Frey *et al.*, 1993; Huang, 1998; Huang and

Kandel, 1994), and both phases are known to be regulated by different cellular signaling pathways (Schulz and Fitzgibbons, 1997). As MMP-9 is found to be a regulator of late-LTP, subsequent studies mainly focused on LTP maintenance. Consequently, a possible contribution of MMP-2 during earlier phases or during short-term potentiation may be overlooked. Interestingly, Western blotting and gelatin zymography show a prominent presence of both pro- and active MMP-2 in cell lysates and culture medium of primary hippocampal neurons (Sbai *et al.*, 2008). In mouse neural crest derived cells, transfected with MMP-2 and MMP-9 constructs fused to GFP, both gelatinases are found in vesicles and co-localized with the molecular motors kinesin and myosin. These vesicles undergo both anterograde and retrograde trafficking along the somato-dendritic compartments and dendritic spines (Fig. 2A,B), serving as an additional indication for an involvement of MMP-2 in synaptic regulation (Sbai *et al.*, 2008). Notably, however, as these studies are performed under non-stimulating conditions, a direct functional evidence for MMP-2 during plasticity is lacking. Nonetheless, MMP-2 is also able to shed the ectodomain of ICAM-5 (Conant *et al.*, 2010; Tian *et al.*, 2007) and to cleave syndecan-2 (Ethell and Yamaguchi, 1999; Fears *et al.*, 2006), β 1-integrin (Ogier *et al.*, 2006), β -dystroglycan (Court *et al.*, 2011) and laminin (Giannelli *et al.*, 1997), relating this gelatinase, together with MMP-9, to dendritic spine morphology (Fig. 2E,G,H). Furthermore, a recently performed MMP-2 degradomics study reveals some putative intracellular substrates (Butler and Overall, 2009) that can be functionally related to plasticity processes, including α -synuclein (Liu *et al.*, 2007) and poly(ADP-ribose) polymerase (Wang *et al.*). Of note, although studies using MMP-2 deficient mice do not reveal alterations in long-term memory or spatial learning (Meighan *et al.*, 2006; Mizoguchi, 2010; Nagy *et al.*, 2006), a defective short-term working memory is noticed in these mice when subjected to a hippocampal-dependent Y-maze task (Mizoguchi *et al.*, 2011).

Overall, in contrast to MMP-9, a clear role for MMP-2 in hippocampal LTP is not yet

determined. However, as several molecules involved in plasticity are already identified as MMP-2 substrates and one *in vivo* study indicates a contribution of MMP-2 in short-term memory processes, an MMP-2 mediated involvement to brain plasticity cannot be totally excluded (see Table 1). Further studies investigating the function of MMP-2 in e.g. hippocampal early LTP or LTD plasticity might provide more detailed information on the role of this gelatinase during hippocampal learning and memory.

3.3 Gelatinase functions during non-hippocampal plasticity in the healthy adult brain

Although MMP-9, and to a more limited extent also MMP-2, are for a long time suggested to be solely involved in hippocampal plasticity, recently, upregulation of these gelatinases is also demonstrated during plasticity processes in other CNS regions (Table 1).

MMP-9 is suggested to underly LTP in the prefrontal cortex (PFC). The PFC receives information from the hippocampus, and LTP in this cortical area is important during e.g. memory integration and decision-making, while its dysfunction results in stress-related phenomena, such as depression and schizophrenia (Akil *et al.*, 1999; Rocher *et al.*, 2004). Treatment of cortical slices with a potent MMP-9 inhibitor (S24994) significantly reduces PFC LTP. Importantly, this MMP-9-dependent late-phase LTP in the PFC is abolished after administration of a TIMP-1 nanobody (Okulski *et al.*, 2007).

Both MMP-2 and MMP-9 are also expressed in neurons of the deep cerebellar nuclei of the adult mouse cerebellum. Environmental enrichment increases gelatinolytic activity and is suggested to promote enhanced synaptic activity by degradation of perineural nets surrounding the deep cerebellar nuclei (Foscarin *et al.*, 2011). These perineural nets, composed of several inhibitory molecules, for example chondroitin sulphate proteoglycans (CSPGs), tenascin-R, hyaluronan and link proteins (Carulli *et al.*, 2006), contain several well-

known gelatinase substrates (Ferguson and Muir, 2000; Nakamura *et al.*, 2000; Turk *et al.*, 2001).

Thus, also in non-hippocampal brain areas, MMP-9 and MMP-2 contribute to neuronal plasticity. However, the general knowledge about MMP function during matrix remodeling in either spine morphology or neurotransmitter receptor activation in non-hippocampal plasticity processes is still insufficient to fully understand the importance of MMPs during plasticity within the entire healthy brain.

4. A ROLE FOR MMP-2 AND MMP-9 IN REGENERATION AND REPAIR OF THE INJURED NERVOUS SYSTEM

A substantial amount of papers already reported the involvement of gelatinases in processes detrimental to the brain. However, a growing number of reports now point to the existence of a so called ‘time window’ in which gelatinases contribute to repair or regeneration of the injured nervous system (see also Table 1 and Fig. 3) (Rivera *et al.*, 2010).

First, important differences in the regenerative capacity of the mammalian CNS and PNS have to be considered. Indeed, in contrast to a fairly robust regeneration of injured nerves in the mammalian PNS, axons in the CNS largely fail to regenerate. This regenerative failure is mainly attributed to (1) the large amount of inhibitory molecules present in the extracellular space of the CNS and in the scar formed by glial cells near the lesion, (2) a low intrinsic regenerative capacity of mature CNS neurons and (3) an insufficient trophic support (Horner and Gage, 2000; Huebner and Strittmatter, 2009). A substantial part of the discrepancy in regenerative potential between the CNS and PNS can probably be assigned to the different myelinating cells present, respectively oligodendrocytes and SCs, as well as to the reduced amount of inflammatory cells that can reach an injured area in the CNS due to its restrictive BBB. Indeed, blood-borne macrophages serve to remove myelin debris after injury, thereby facilitating axonal regrowth and remyelination (Cui *et al.*, 2009). Finally, also resident glial

cells cannot be excluded from our discussion since recent publications report a role for these cells in regeneration and repair of the PNS and CNS (see below).

A variety of MMP expression studies indicate gelatinases as promising candidates for both PNS and CNS regeneration, in animals exhibiting spontaneous regeneration of their nervous system after injury. Here, MMP-2 and MMP-9 levels are reported to be upregulated at various stages of nerve regeneration (Chernoff *et al.*, 2000; McCurley and Callard, 2010). In axolotls, for example, MMP-2 and MMP-9 are highly upregulated during spinal cord regeneration, while gelatinase activity decreases as regeneration proceeds (Chernoff, 1996; Chernoff *et al.*, 2000). RT-PCR and microarray studies performed in the regenerating zebrafish eye at different time points after optic nerve crush, show a promising correlation between MMP expression and regeneration. Indeed, MMP-9 levels are increased during the injury-response phase and both MMP-9 and MMP-2 expression augments in the axonal outgrowth phase. Also here, gelatinase expression decreases near completion of regeneration (McCurley and Callard, 2010). Finally, even in mammals, the temporary and restricted neurite growth into scar tissue of the damaged adult spinal cord coincides with a strong induction of gelatinase activity, which seems necessary to form migratory pathways through the inhibitory environment along which neurites grow (Duchossoy *et al.*, 2001a; Duchossoy *et al.*, 2001b). Notably, in the PNS, more specifically after sciatic nerve crush, MMP-9 is upregulated in a dual mode: not only at 6h after lesioning, but also during the regenerating phase at 2 and 3 weeks post-injury (Hughes *et al.*, 2002; Platt *et al.*, 2003; Shubayev and Myers, 2000, 2002).

Both MMP-2 and MMP-9 are also known or suggested to be implicated in many processes contributing to a successful neuronal regeneration, i.e. in post-injury de- and remyelination, in inflammation and glial reactivity, in axonal outgrowth/elongation and synapse formation, in neural progenitor cell genesis and migration, and in angiogenesis. However, depending on the type of nervous system, MMP-2 and MMP-9 seem to regulate

different processes. Therefore, in the following paragraphs we will provide a summary of the wide variety of functions these gelatinases exert in regeneration and repair of the PNS and the CNS.

4.1 Functions of MMP-2 and MMP-9 in the early injury response of the PNS

Soon after PNS injury, denervated myelinating SCs start proliferating, release cytokines and phagocytose detached debris. This vigorous response of non-neuronal cells present in the distal nerve leads to degeneration of the detached distal axons in a process named ‘Wallerian degeneration’. As Wallerian degeneration is well on its way, activated resident and recruited hematogenous macrophages clear myelin and axon debris and produce factors that facilitate SC migration and axonal regeneration (Gaudet *et al.*, 2011) (Fig. 3A-D). Both gelatinases seem mainly involved in macrophage infiltration but MMP-9 has also been reported to affect SC regulation (see below) (Fig. 3B,C). By subjecting rat sciatic nerves to a chronic constriction injury, Shubayev and Myers show co-localization of TNF- α with MMP-2 in endothelial cells and with MMP-9 in macrophages during active immune cell migration. Since MMP-2 can promote the degradation of blood vessel endothelium, resulting in an increased vascular permeability, and because MMP-9 is known to play a role in cell migration, it is suggested that macrophage recruitment from the circulation is directly dependent on MMP-2 and -9, both regulated by the cytokine TNF- α (Shubayev and Myers, 2000, 2002) (Fig. 3B). Notably, also after transection of the mouse sciatic nerve, elevated MMP-2 and -9 activities coincide with blood-nerve barrier breakdown and macrophage invasion. Furthermore, intraperitoneal injections of hydroxamate, a nonspecific MMP inhibitor, delays Wallerian degeneration, by decreasing macrophage invasion into the sciatic nerve, again suggesting a role for gelatinases in macrophage recruitment into the damaged peripheral nerve (Siebert *et al.*, 2001).

Besides its involvement in macrophage infiltration, MMP-9 has also been implicated in the SC phenotypic remodeling process that occurs after injury of the PNS. SC phenotypic remodeling involves dedifferentiation, proliferation and migration of SCs, leading to formation of bands of Bungner, rail-track-like structures upon which axons can efficiently regenerate (Chattopadhyay and Shubayev, 2009; Triolo *et al.*, 2006) (Fig. 3C). A strict regulation of this phenotypic remodeling is thus essential to obtain a successful regeneration of damaged peripheral nerves. By comparing BrdU incorporation in axotomized sciatic nerves of wild-type and MMP-9 deficient mice and by treatment of a primary SC culture with rMMP-9, it was shown that MMP-9 suppresses the mitogenic activity of SCs. This inhibitory action seems to reduce excessive proliferative activity and thus abnormal remyelination patterns (Chattopadhyay and Shubayev, 2009; Kim *et al.*, 2012). MMP-9 is suggested to activate the Ras/Raf/mitogen-activated protein kinase (MEK) - extracellular-signal-regulated kinase (ERK) pathway, involved in cell cycle arrest of SCs, via neuregulin/ErbB, IGF-1 and PDGF ligand/receptor signaling cascades (Chattopadhyay and Shubayev, 2009; Kim *et al.*, 2012; Mantuano *et al.*, 2008). Besides induction of SC differentiation, MMP-9 also promotes SC migration after injury (Fig. 3C), most likely via the low-density lipoprotein receptor-related protein (LRP-1) receptor, which is only upregulated by SCs after PNS lesions (Campana *et al.*, 2006; Mantuano *et al.*, 2008). Indeed, by inhibiting MMP-9 binding to LRP-1 through LRP-1 gene silencing or administration of blocking antibodies targeting the MMP-9 hemopexin domain, migration of cultured rat sciatic nerve SCs is reduced. Notably, a fusion protein that only contains the hemopexin domain of MMP-9 (MMP-9-PEX) is able to replicate MMP-9 actions and thus to activate SC migration. Moreover, as constitutively active MEK1 mimics the actions of MMP-9-PEX, it is proposed that MMP-9 also influences SC migration via activation of ERK1/2 (Mantuano *et al.*, 2008).

Thus, both gelatinases seem important beneficial regulators during the early phase,

injury response, in the damaged PNS. They are both involved in macrophage recruitment, while MMP-9 also seems to regulate SC reactivity after injury.

4.2 Functions of MMP-2 and MMP-9 in the early injury response of the CNS

Compared to the PNS, CNS injury only results in a temporary axonal sprouting response, followed by axonal degeneration and ultimately even neuronal cell death (Fig. 3E-H). This can be partly explained by the fact that, in contrast to SCs, oligodendrocytes only provide a minimal growth support and phagocytic activity of tissue debris after axonal injury and even tend to undergo apoptosis or enter a quiescent stage (Barres *et al.*, 1993; Crowe *et al.*, 1997; Fawcett, 2006; Yiu and He, 2006). Moreover, also the limited number of resident and blood-borne macrophages present at the site of injury supplies an insufficient clearance of tissue debris and inhibitory ECM molecules, thereby contributing to failure of axonal regeneration (Fig. 3F). Besides this excess of inhibitory molecules, also the glial scar, which is produced by reactive astrocytes, forms an impenetrable barrier for outgrowing axons (Fig. 3G) (Horner and Gage, 2000; Huebner and Strittmatter, 2009). Despite the beneficial functions of a glial scar, e.g. reconstitution of the BBB and restriction of neuronal degeneration after CNS injury (Kawano *et al.*, 2012), it contains many molecules inhibitory to axonal regeneration, such as CSPGs (Fig. 3G). A considerable number of studies thus focused on elucidating mechanisms involved in degradation of these inhibiting molecules and gelatinases are repeatedly implicated as promising molecules in this matter. Indeed, in a rat regenerating optic nerve crush model, upregulated MMP-2 and -9 activity can be observed in astrocytes, present at the proximal optic nerve stump and around the lesion site, and therefore gelatinases are proposed to contribute to the breakdown of inhibitory molecules, thereby clearing the path for axonal sprouting and regeneration (Ahmed *et al.*, 2005) (Fig. 3I). Furthermore, a crucial role for MMP-2 in limiting glial scar formation by proteolytic

degradation of CSPGs has been repeatedly suggested. Indeed, MMP-2 deficient mice show an impaired structural and functional recovery after spinal cord injury due to an increased glial scarring (Hsu *et al.*, 2006). Secondly, MMP-2 is clearly upregulated in olfactory ensheathing cell (OEC) grafts transplanted into damaged rat spinal cords and would thus support the capacity of OECs to promote adult CNS axon regeneration, most likely by cleavage of CSPGs, since the amount of CSPGs present in scar tissue strongly decreases (Fig. 3I) (Pastrana *et al.*, 2006). Furthermore, injured rat spinal cords show improved functional recovery after transplantation with human umbilical cord blood mesenchymal stem cells (hUCBs), known to induce and maintain tissue repair. This recovery is most likely due to an evoked MMP-2 upregulation in spinal cord neurons adjacent to the site of injury, which in turn reduces the formation of a glial scar (Veeravalli *et al.*, 2009). Moreover, also immature astrocytes, which have the ability to cross proteoglycans (Filous *et al.*, 2010), thereby suppressing glial scar formation (Smith and Miller, 1991), would exert their effects via MMP-2 and its capacity to degrade CSPGs. Indeed, addition of an MMP-2 inhibitor I (inhibits MMP-2 in a dose-dependent manner) to immature astrocytes, which inhibits MMP-2 in a dose-dependent manner, plated on a spot gradient that imitates the inhibitory proteoglycans present in the glial scar, strongly reduces the number of astrocytes able to cross the most inhibitory outer proteoglycan rim and the potential of axon regrowth (Filous *et al.*, 2010).

Finally, MMP-2 and -9 are also suggested to be indirectly involved in the reduction of scar tissue after CNS injury, more specifically by influencing the migration of cells known to promote regeneration (Fig. 3I). Indeed, in transplanted OECs, which are migrating through the scar tissue of rodents suffering from spinal cord injury, MMP-2 and MMP-9 are proteolytically active and distributed along microtubules and microfilaments where they co-localize with the motor protein kinesin. Increased gelatinase activity is also noted during OEC migration *in vitro*, further confirming their involvement in OEC migration. Moreover,

inhibition of MMP-2 is associated with a defective OEC migration on laminin and collagen, typical substrates for regenerating nervous tissue (Gueye *et al.*, 2011).

In summary, despite a frequently reported detrimental role for MMP-2 and -9 in damaged neuronal tissue, gelatinases are recently brought forward as potential benefactors in the early phase of regeneration of the nervous system, even in the CNS. Here, mainly MMP-2 has been repeatedly proposed as a regenerative factor, as this enzyme is able to cleave CSPGs and thus to reduce the formation of the glial scar. Notably, until now, gelatinases are not assumed to be involved in neuroprotection after injury, but are rather reported as cell death promoting factors (Chaudhry *et al.*, 2010; Lee *et al.*, 2004).

4.3 A role for MMP-2 and MMP-9 in axonal outgrowth after damage in the PNS/CNS

After assuring clearance of the inhibitory environment and survival of neuronal cells in the injured area, a next step for successful regeneration within the nervous system is axonal sprouting and outgrowth. Both MMP-2 and -9 are suggested to promote axonal outgrowth, respectively in the CNS and PNS, by regulating different underlying mechanisms. MMP-9 is found to co-localize with the growth-associated protein GAP-43 and observed in the axoplasm of myelinated regenerating fibers of crushed sciatic nerves (Fig. 3C) (Kim *et al.*, 2012; Shubayev and Myers, 2004). Addition of rMMP-9, a neutralizing anti-MMP-9 antibody or a broad-spectrum MMP inhibitor to cultured rat pheochromocytoma (PC12) cells treated with NGF, used as a model for neuronal sprouting, revealed that MMP-9, does not affect the formation of sprouts, but is involved in subsequent axonal elongation and branching. These results suggest that MMP-9 might be an important regulator of neurite extension in regenerating peripheral nerve fibers (Fig. 3C) (Shubayev and Myers, 2004). In contrast, in the CNS, MMP-2 is believed to promote axonal outgrowth of adult rat RGCs by digestion of PNNs (Fig. 3I). Indeed, in the presence of active MMP-2, regenerating retinal neurons plated

on OEC monolayers show reduced PNNs. Moreover, functional silencing of MMP-2 decreases the capacity of these cultured neurons to regrow their axons (Pastrana *et al.*, 2006).

Overall, studies highlighting a possible beneficial role for MMP-9 or MMP-2 in axonal sprouting/outgrowth/elongation after PNS or CNS injury are still elusive. Future research efforts are urgently needed to confirm or disprove previous findings.

4.4 MMP-9 functions in PNS and CNS remyelination

Remyelination is an important process during nerve repair and regeneration in the PNS and CNS and has been associated with MMP-9, but not with MMP-2. Notably, remyelination in the PNS clearly differs from remyelination in the CNS. In the PNS, the MMP-9/TIMP-1 axis is proposed to play an essential role in guiding the differentiation of myelinating SCs and the subsequent remyelination during sciatic nerve repair in mice (Kim *et al.*, 2012) (Fig. 3B,C). Indeed, as MMP-9, expressed in myelinating SCs of injured sciatic nerves, has anti-mitogenic activity on SCs within the first days after rat sciatic nerve crush, it also determines the amount of post-mitotic SCs in regenerating nerves (Chattopadhyay and Shubayev, 2009; Kim *et al.*, 2012). Importantly, because every myelinating SC forms a myelin internode, the very robust proliferation of myelinating SCs after injury in MMP-9 deficient mice leads to a significantly reduced internodal length of remyelinating sciatic nerves, measured by immunostainings for laminin-2. Yet, significant abnormalities in motor nerve conductions were not detected, probably due to functional redundancy by other MMP family members (Kim *et al.*, 2012). Notably, laminin-2 is not only a component essential for establishing the proper internodal length, but is also a potential substrate of MMP-9. In addition, MMP-9 likely promotes the post-mitotic maturation of SCs, since a higher number of GFAP⁺/myelin basic protein (MBP)⁺ cells is found in crushed sciatic nerves of MMP-9 deficient mice (Kim *et al.*, 2012). Knowing that the SC basement membrane is composed of laminins, which are

involved in SC morphogenesis and myelin ensheathment (Chernousov *et al.*, 2008; Court *et al.*, 2006) and susceptible to MMP-9 proteolysis (Gu *et al.*, 2005), it seems logic to hypothesize that MMP-9 may control SC maturation and myelin ensheathment (Fig. 3C) through proteolysis of laminins (Kim *et al.*, 2012).

In the CNS, various types of demyelinating insults may lead to permanent dysfunctions, as remyelination can only occur to a limited extent. As such, many studies already focused on elucidating the mechanisms underlying remyelination in the CNS. Since migration and maturation of oligodendrocyte precursors and the ensheathment of axons by their processes form an essential part of the remyelinating event and need extensive ECM remodeling, proteases, and MMPs in particular, have been suggested as promising candidate molecules herein (Yong, 2005). Indeed, MMP-9 deficient mice, subjected to a lysolecithin-induced demyelinating injury of the spinal cord, show an impaired remyelination capacity as compared to wild-type animals. Moreover, MMP-9 deficiency results in less mature oligodendrocytes in the lesion area, probably caused, in part, by the failure to clear NG2 CSPG deposits. These inhibitory proteoglycans, which are well known substrates of MMP-9, presumably retard the maturation and differentiation of oligodendrocytes (Larsen *et al.*, 2003). Although conclusive evidence is limited, these findings suggest a role for MMP-9 in remyelination of the injured CNS (Fig. 3I).

Overall, in the PNS, MMP-9 seems to regulate the molecular and structural assembly of myelin sheaths by regulating myelinating SC proliferation and maturation, while in the CNS, modulation of the ECM by MMP-9 is necessary to support oligodendrocyte maturation.

4.5 A role for MMP-2 and MMP-9 in post-injury synaptic remodeling?

A successful regeneration after neuronal injury concomitant with axonal degeneration, implies the generation of new synapses in order to regain neuronal functionality. As

synaptogenesis depends on dynamical changes of the ECM and cleavage of pericellular protein-like growth factors and adhesion molecules, a role for MMP-2 in this elaborate process has been proposed, but only limited evidence has been gathered. As such, this gelatinase seems to play a key function in the reinnervation and synaptic remodeling of the cochlear nuclear complex in the auditory brainstem, triggered by unilateral cochlear ablation. Here, MMP-2 is secreted by neurons and adjoining astrocytes and its accumulation coincides with the emergence of GAP-43 positive presynaptic terminals (Fredrich and Illing, 2010). Clearly, conclusive proof for a role of the gelatinases in *de novo* synaptogenesis after injury is poor and further research should provide more insight into their contribution within this process.

Additionally to *de novo* synaptogenesis of newly regenerated axons, reorganization of synaptic contacts from neighbouring axons also occurs, defined as post-injury synaptic plasticity, a process in which MMP-9 has recently been implied. Indeed, enhanced synaptic activity and subsequent tissue remodeling, triggered by kainate administration, results in an upregulation of MMP-9 mRNA, protein and activity in the adult rat hippocampal dentate gyrus at the site of granule neuron dendrites, implicating a role for MMP-9 in activity-dependent remodeling of dendritic architecture (Szklarczyk *et al.*, 2002). Also in the adult mouse barrel cortex, MMP-9 is suggested to regulate plasticity, evoked by sensory deprivation induced by eliminating all rows of vibrissae except one. As a result, the functional representation of the spared row of whiskers significantly increases, expanding to the adjacent deprived barrel cortex and thus leading to remapping of cortical topography. Broadening of the functional representation of the spared row of vibrissae coincides with a higher MMP-9 activity in layer IV of the deprived barrel cortex after exploratory behavior and is highly reduced in MMP-9 deficient mice (Kaliszewska *et al.*, 2011). It is assumed that MMP-9 directly influences functional representation, presumably via the plasticity-promoting

mechanisms observed in the hippocampus during LTP (see section 3.1) (Kaliszewska *et al.*, 2011). Notably, the involvement of MMP-9 during enhanced neuronal activity is also believed to be mediated by IGF binding protein-3 cleavage in the brain endothelium, promoting serum IGF-1 uptake via the BBB into the CNS (Nishijima *et al.*, 2010). Furthermore, MMP-9 seems to be involved in lesion-induced sprouting of intact retinal axons during the critical period in rats (Penedo *et al.*, 2009). Serotonin is a well known modulator of axon reorganization in the retinotectal pathway after retinal lesions (Bastos *et al.*, 1999), and nutritional restriction of tryptophan, which decreases brain serotonin levels, indeed results in a marked reduction of axonal plasticity. This finding correlates with a decreased MMP-9 activity in superior colliculus extracts of tryptophan restricted rats and thus associates reduced MMP-9 activity with a decrease in plasticity (Penedo *et al.*, 2009). Additionally, these results suggest a serotonergic-dependent modulation of the ECM via MMP-9, possibly through the MMP-9-induced conversion of pro-BDNF to mature BDNF, resulting in TrkB receptor activation (Hwang *et al.*, 2005) and subsequent increased plasticity (Penedo *et al.*, 2009).

Thus, a role for gelatinases in *de novo* synaptogenesis remains poor. Even though, multiple findings already indicate a key role for MMP-9 in post-injury synaptic remodeling. In accordance to the findings for both gelatinases in healthy adult brain plasticity, summarized above (see section 3), additional MMP-2/9 research on synaptogenesis and plasticity during the post-injury phase seems definitely required.

4.6 A role for the gelatinases in adult neurogenesis, neuroblast migration and angiogenesis

Next to axonal regeneration of surviving cells in the damaged nervous system, time- and place-related loss of cells should ideally be compensated and supported by generation of new, functionally active neurons. Yet, this process is very restricted in the adult mammalian CNS. As neuroblast migration requires remodeling of the ECM, a contribution of gelatinases

to neurogenesis and subsequent migration of newly generated neurons from the subventricular zone (SVZ) and hippocampal dentate gyrus towards injured brain areas, for instance after stroke, has been suggested (Kang *et al.*, 2008; Lee *et al.*, 2006; Wang *et al.*, 2006). Additionally, MMP-2 and MMP-9 are believed to support angiogenesis in the adult (injured) brain, as both gelatinases are known and previously mentioned to be expressed by endothelial cells during angiogenesis in physiological and pathological conditions. Moreover, neuro- and angiogenesis are closely related as blood supply is essential for neuronal survival/development and common mechanisms underlie both processes (Itoh *et al.*, 1998; Nguyen *et al.*, 2001; Slevin *et al.*, 2006).

In vitro studies, in which adult neural progenitor cell (aNPC) differentiation and migration is evoked by using injury-induced chemotaxic factors, show upregulated MMP-9 mRNA and protein levels, while MMP-9 knock-down in this assay interrupts aNPC proliferation and differentiation (Barkho *et al.*, 2008). Also, when mouse brain endothelial cells, cocultured with aNPCs from the SVZ, are activated by erythropoietin, they promote aNPC migration while simultaneously upregulating mRNA levels and pro and active forms of MMP-2 and MMP-9 (Wang *et al.*, 2006). Furthermore, during the recovery phase in rodent stroke models, MMP-9 mRNA and protein expression is found in aNPCs migrating from the SVZ towards the ischemic zone (Barkho *et al.*, 2008; Lee *et al.*, 2006). Also in the adult gerbil, forebrain ischemia results in an increased proliferation of aNPCs in the dentate gyrus and is accompanied by elevated gelatinase-activity (Wojcik *et al.*, 2009). In a rat stroke model, elevated MMP-9 expression is detected in neurons and astrocytes at the peri-infarct cortical areas at later stages after focal cerebral ischemia and colocalizes with neurovascular remodeling markers (Zhao *et al.*, 2006). Upregulated MMP-9 is also found in astrocytes in the peri-infarct mouse cortex after photothrombic ischemia (Kang *et al.*, 2008). Treatment with broad-spectrum MMP inhibitors (FN-439 or BB-94) impairs neurovascular remodeling and

functional repair and increases ischemic brain injury, indicating the importance of MMPs, especially MMP-9, in stroke recovery (Kang *et al.*, 2008; Zhao *et al.*, 2006).

A link between MMP-2, MMP-9 and adult neurogenesis is also reported in macaque monkey after transient global brain ischemia. Using gelatin zymography, a biphasic upregulation of MMP-9 levels is found, first in the acute deleterious phase and next also in the delayed, repair phase of the post-ischemic dentate gyrus. In contrast, MMP-2 is only upregulated during the delayed phase. During this delayed phase, immunohistochemical stainings reveal MMP-2 and MMP-9 expression in endothelial cells, astrocytes and newborn neurons of the subgranular zone of the dentate gyrus, which suggests their involvement in angiogenic and neurogenic pathways (Lu *et al.*, 2008). Also in adult songbirds, the steroid-induced neurogenesis and angiogenesis in the vocal control nucleus designated HVC (hyperstriatum ventrale, pars caudalis) are proposed to be regulated by endothelial MMP-2 (Kim *et al.*, 2008). Notably, the testosterone-triggered, VEGF-induced release of MMP-2 from endothelial cells seems to modulate neuronal recruitment into the HVC. In addition, the subsequent MMP-2-mediated release of matrix-bound VEGF amplifies VEGF levels, thereby stimulating the androgenic induction of angiogenesis (Kim *et al.*, 2008).

From all these observations, one can appreciate the critical role of MMP-2 and MMP-9 in the correlated processes of angiogenesis and neurogenesis at neurogenic niches.

5. CONCLUSIONS

MMPs are implicated in many diseases and neurological disorders, and are therefore suggested to be promising targets for new drug therapies. Yet, importantly, as MMPs each contain a large degradome and thus a broad range of biological functions, various criteria need to be considered when validating MMPs as drug targets. As such, it is of utmost importance to acquire additional knowledge on their beneficial properties. Their specific spatial and temporal regulation in a given process have to be considered. This review highlights the diverse roles of two extensively studied MMPs, MMP-2 and MMP-9, during nervous system development and adult plasticity, and implies the importance of gelatinase upregulation to improve post-injury repair of the nervous system.

During nervous system development, MMP-2 contributes to neovascularization, thereby assuring a sufficient blood supply in the developing brain. Furthermore, the well-defined spatiotemporal upregulation and activation observed during development propose a role for both gelatinases in neurogenesis, neuron/astrocyte migration, neurite outgrowth and myelination. However, as functional studies are limited, knowledge on the exact working mechanisms remains largely elusive. In contrast, during plasticity processes in the normal adult brain, MMP-9 is extensively studied, in particular in hippocampal learning-dependent LTP, and is identified as an important regulator of late-phase LTP by activating NMDARs and influencing dendritic spine morphology. A contribution of MMP-2 during short-term memory or non-hippocampal plasticity is not unlikely, but needs to be further investigated. Finally, MMP-2 and MMP-9 seem important candidate molecules in supporting regeneration events in the damaged adult nervous system. Several MMP-2/-9 substrates underlying regenerative processes have already been identified, and their involvement in neurogenesis and in the early phase after injury, for example during macrophage infiltration, demyelination and CSPG breakdown, is well established. Nonetheless, during the late post-injury response, a

contribution of the gelatinases in remyelination, axonal outgrowth or synaptogenesis, remains rather elusive. Several *in vivo* validation experiments and proteomic studies are commended to gain increasing insight into the underlying working mechanisms. Importantly, since the regenerative events resemble to some extent developmental processes and adult plasticity-dependent synaptic remodeling, in which the gelatinases, and in particular MMP-9, are known to play a crucial role, future in depth investigations will presumably provide the necessary information confirming the beneficial contribution of MMP-2 and/or MMP-9 in post-injury repair of the nervous system.

In conclusion, and as summarized in table 1, the beneficial roles of the gelatinases in nervous system development, plasticity and post-injury repair are unambiguous. Keeping in mind the current use of largely non-specific MMP inhibitors due to cross-reactions with other MMPs, a more precise inhibition of MMP-2 and MMP-9 activity is needed to further investigate their independent and spatiotemporal contributions in specific processes. This can be obtained by using conditional and/or cell-specific transgenic animals or RNAi-mediated MMP downregulation, by employing highly specific MMP-2 and MMP-9 function blocking inhibitors or antibodies/nanobodies, such as the recently described single function MT1-MMP inhibitor, which specifically targets MMP-2 activation (Ingvarsen *et al.*, 2013). These approaches will undoubtedly uncover important time and space restricted MMP functions and will be highly valuable for designing future therapeutic strategies. Nonetheless, given the elaborate MMP degradome and their involvement in many physiological and detrimental conditions, clarifying the exact working mechanisms underlying MMP proteolysis remains very challenging. Degradomic studies in transgenic animals or after genetic or pharmacological MMP inhibition, and additional use of mice deficient in particular MMP substrates could give valuable information about putative downstream pathways. Still, additional direct approaches, such as *in vitro* cleavage or yeast-two-hybrid assays, are needed

to unravel direct MMP substrates. Furthermore, experiments using mutagenesis of potential cleavage sites or cleavage site protection by application of antibodies or peptides binding to the cleavage sites, will provide additional knowledge on the pathways involved. These studies are highly lacking today, but will certainly be essential to understand the whole story on MMP-mediated proteolysis in the CNS and beyond.

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Fig. 1 Functional implications for MMP-2 and -9 during nervous system development

MMP-2 and MMP-9 are suggested to be important in a variety of developmental processes within the nervous system. During neovascularization, MMP-2 is mainly expressed in pericytes and is able to cleave the collagen type IV-rich basement membrane (A). Hereby, endothelial vessel sprouting can be initiated. Furthermore, during neurogenesis, MMP-9 seems important in NSC proliferation, while MMP-2 is implicated in neural crest cell differentiation, leading to a correct formation of different cell types, including neurons and oligodendrocytes (B). Hereafter, different neuronal cell types migrate to their final position, which is also believed to be influenced by gelatinases, e.g. by controlling β 1-integrin signaling via ectodomain shedding, or by directly modulating cytoskeletal components, such as actin, intracellularly (C). MMP-2 and MMP-9 are also involved in axonal and dendritic outgrowth and guidance (D). At the dendrites (D, left panel), they are able to cleave ICAM-5, leading to a correct dendritic outgrowth via cell-to-cell adhesion and interference of ICAM-5 with the cytoskeleton through interactions with ERM family proteins. Additionally, MMP-2 is activated upstream by PKC α , after binding of Sema3A to the NRP1 receptor, thereby supporting dendritic outgrowth. Besides, at the growing tips, extracellular MMP-2 is responsible for the conversion of pro-NGF to its mature form, which binds to the TrkA receptor and subsequently leads to nerve sprouting. At the axon (D, right panel), pro-MMP-9 is upregulated after FGFR activation through FGF-2 binding. FGFRs induce neurite outgrowth by interacting with NCAM, which is in turn a known substrate of MMP-9. At the growth cone, MMP-9 can regulate axonal guidance through EphB2 cleavage when associated with its ligand EphrinB2 on a neighbouring cell/growth process, resulting in cell-cell repulsion via cytoskeletal responses. Finally, both gelatinases have been described to regulate oligodendrocyte (MMP-9) and Schwann cell (MMP-2) differentiation, mainly during the early phase of myelination formation (E).

Fig. 2 Functional implications for MMP-2 and -9 in synaptic plasticity

The gelatinases, and in particular MMP-9, are known to be involved in regulation of synaptic plasticity, mainly during hippocampal learning and memory. mRNA of both proteinases are believed to be transported through vesicles along the somato-dendritic compartment (A), whereafter translation occurs at the dendritic spine (B). MMP-2 and MMP-9 can act intracellularly, but are mainly secreted to the ECM where they can be activated (C). MMP-9 (and possibly also MMP-2) is able to influence Ca^{2+} influx postsynaptically by indirectly activating LVDCCs and NMDARs. MMP-9 affects LVDCC activation by processing ECM molecules that are in complex with these LVDCCs, such as tenascin-C (D). Increased Ca^{2+} currents are induced after NMDAR trafficking (dashed arrow) and subsequent activation through NMDAR phosphorylation (E). These mechanisms are influenced by β -dystroglycan and EphB2, substrates of MMP-9 and/or MMP-2 (F), but are mainly believed to be affected by β 1-integrin signaling, for example through binding of the soluble ICAM-5 ectodomain, after cleavage by MMP-2 and -9 (G). Besides, MMP-2 and MMP-9 are also believed to alter spine morphology during plasticity, for example through laminin cleavage, whereafter Arg-Gly-Asp (RGD) recognition sites on laminin become available for binding to β 1-integrin receptors (H). The integrin signaling pathway in turn influences actin polymerization, leading to alterations in spine morphology, e.g. growth of spine heads. Moreover, also microtubule assembly is necessary for spine growth and might be influenced by CRMP-2 through MMP-9-dependent activation at the postsynaptic site (I). Presynaptically, this MMP-9-mediated CRMP-2 activation also increases glutamate release from vesicles, after binding of active CRMP-2 to a voltage-dependent Ca^{2+} channel (Cav2.2) (J). Finally, synapse functionality through formation of transsynaptic adhesion complexes is also suggested to be regulated by MMP-9 dependent cleavage of SynCAM-2, a MMP-9 target (K).

Fig 3. Functional implications for MMP-2 and -9 in PNS and CNS regeneration

Although intact neurons of the PNS and CNS have a comparable morphology (A,E), injured peripheral neurons have a far more greater capacity to regenerate in comparison to damaged CNS neurons (D,H). In the PNS, gelatinases seem to contribute at different levels to this high regenerative capacity. Indeed, shortly after damage, upregulated levels of MMP-2 and -9, expressed respectively by endothelial cells and hematogenous macrophages, facilitate the infiltration of activated macrophages which, in association with activated resident microglia and SCs, phagocytose myelin and axon debris, thereby clearing the path for regrowing axons (B). In the injured PNS, MMP-9 is expressed by SCs and controls SC proliferation, maturation (B) and migration (C), thereby supporting the formation of bands of Bungner (arrowheads) (C). Finally, MMP-9 expression is also observed in regenerating peripheral nerve fibers and is suggested to promote axonal outgrowth in the injured PNS (C), thereby contributing to a successful regeneration in the PNS (D). However, in the CNS, failure of axonal regeneration is partly due to a more limited blood-borne macrophage infiltration (F), resulting in a reduced ability to clear the inhibitory environment, i.e. myelin debris and a glial scar consisting of CSPGs formed by reactive astrocytes (G). In addition, oligodendrocytes show a limited phagocytic activity and growth promoting response, and enter a quiescent state or even undergo apoptosis (G). Together with the low intrinsic regenerative capacity of mature CNS neurons and an insufficient trophic support, this will trigger axonal degeneration and consequently, neuronal cell death (G,H). However, recent findings indicate that limited CNS regeneration can be initiated and also gelatinases are increasingly being recognized as important contributors in supporting recovery from CNS injuries (I). Expression of these proteinases by hUCBs, astrocytes and OECs seems to promote reduction of the glial scar and to support axonal regrowth through cleavage of CSPGs (I). Moreover, MMP-2 is believed to enhance axonal outgrowth by e.g. PNN cleavage (I) and both MMP-2 and -9 are reported to

indirectly reduce the glial scar by influencing the migration of various cells able to promote regeneration, like OECs. Finally, MMP-9 might improve CNS remyelination by cleavage of NG2 deposits, which prevent oligodendrocyte maturation (I).

Table 1

Overview of neuronal processes linked to MMP-2 or -9 and their substrates during nervous system development, adult plasticity processes and regeneration after injury

Process	MMP	Tissue/cellular localization	(Putative) contribution	Known/suggested substrates	References
Development^a					
<i>Neovascularization</i>	MMP-2	telencephalon (pericytes)	Initiation vessel sprouting BBB differentiation	Laminin, fibronectin and type IV collagen-rich basement membrane	(Girolamo <i>et al.</i> , 2004) (Virgintino <i>et al.</i> , 2007)
	MMP-2	Cornea	Degradation vascular basement membrane/endothelial proliferation	type IV collagen	(Kato <i>et al.</i> , 2001) (Murphy <i>et al.</i> , 1993)
<i>Neurogenesis</i>	MMP-9	Neural stem cells	NSC proliferation via Wnt/ β -catenin signaling pathway	Unknown	(Ingraham <i>et al.</i> , 2011)
<i>Differentiation</i>	MMP-9	Neural crest-derived cells	Transition to neuronal-like cells	Unknown	(Higgins <i>et al.</i> , 2009)
<i>Neuroprotection</i>	MMP-9	Cultured hippocampal neurons	Regulation of developmental neuronal apoptosis	Laminin	(Murase and McKay, 2012)
<i>Migration</i>	MMP-2	Neural crest-derived cells	Contribution to migration-induced mesenchymal transition	Unknown	(Duong and Erickson, 2004)
	MMP-2/-9	Cerebellar granule cells	Granule cell radial migration	Unknown	(Ayoub <i>et al.</i> , 2005) (Vaillant <i>et al.</i> , 2003)
	MMP-2/-9	Astrocytes	Cytoskeleton alterations	β -1 integrin Vitronectin	(Ogier <i>et al.</i> , 2006) (Faber-Elman <i>et al.</i> , 1995) (Overall, 2002)
	MMP-2/-9	Olfactory epithelium cells/neuroblastoma cells	Cytoskeletal interactions	Unknown	(Gueye <i>et al.</i> , 2011) (Sbai <i>et al.</i> , 2008)
<i>Dendritic outgrowth and guidance</i>	MMP-2	Apical cortical dendrites	Dendritic outgrowth in response to Sema3A via PKC induction	Unknown	(Gonthier <i>et al.</i> , 2009)
	MMP-2	Sympathetic neurons	Nerve sprouting	NGF	(Saygili <i>et al.</i> , 2011)

	MMP-2/-9	Vesicles in hippocampal neuron dendrites	Dendritic elongation	ICAM-5	(Tian <i>et al.</i> , 2007)
<i>Axonal outgrowth and guidance</i>	MMP-9	Cerebellar granule cells	Axonal outgrowth in tangentially migrating granule cells	Unknown	(Vaillant <i>et al.</i> , 2003)
	MMP-9	HEK293 cells + cultured hippocampal neurons	Cell-cell repulsion	EphB2R	(Lin <i>et al.</i> , 2008)
	MMP-9	Cultured neuroblastoma cells/cortical neurons	Axonal outgrowth and guidance	NCAM	(Niethammer <i>et al.</i> , 2002) (Fujita-Hamabe and Tokuyama, 2012)
<i>Oligodendrocyte differentiation and myelination</i>	MMP-9	Spinal cord , corpus callosum, optic nerve: around oligodendrocyte processes	Oligodendrocyte maturation and process extension	MBP	(Liu <i>et al.</i> , 2012) (Uhm <i>et al.</i> , 1998) (Oh <i>et al.</i> , 1999)
	MMP-2	Schwann cells	Early-phase myelination of DRG cells co-cultured with Schwann-cells	Unknown	(Lehmann <i>et al.</i> , 2009)
Plasticity^b <i>Hippocampal</i>	MMP-9	Hippocampal excitatory dendritic spines	LTP maintenance :	sAPPα	(Nagy <i>et al.</i> , 2006) (Fragkouli <i>et al.</i> , 2012)
			LVDCC regulation (Ca ²⁺ influx)	Tenascin-C	(Strekalova <i>et al.</i> , 2002) (Evers <i>et al.</i> , 2002) (Kalembeiyi <i>et al.</i> , 2003)
			NMDAR trafficking	β-1 integrin	(Groc <i>et al.</i> , 2007) (Michaluk <i>et al.</i> , 2009)
				β-dystroglycan EphB2R	(Michaluk <i>et al.</i> , 2007) (Lin <i>et al.</i> , 2008) (Takasu <i>et al.</i> , 2002)
			Altering spine morphology	CRMP-2 SynCAM-2	(Wang <i>et al.</i> , 2008), (Kramar <i>et al.</i> , 2006) (Michaluk <i>et al.</i> , 2011) (Bajor <i>et al.</i> , 2012)
	MMP-2/-9	Hippocampal excitatory neurons: nuclear localization	Altering spine morphology	ICAM-5	(Sbai <i>et al.</i> , 2008) (Tian <i>et al.</i> , 2007), (Conant <i>et al.</i> , 2010)

	MMP-2	Hippocampal excitatory neurons	Hippocampal short-term memory	Unknown	(Mizoguchi, 2010)
<i>Non-hippocampal</i>	MMP-9	Prefrontal cortex (PFC)	Late-phase PFC LTP	Unknown	(Okulski <i>et al.</i> , 2007)
	MMP-2/-9	DCN neurons	Enhancing neuronal activity and transmission after environmental enrichment	PNN components	(Foscarin <i>et al.</i> , 2011)
<i>Regeneration^c</i>					
<i>PNS regeneration</i>	MMP-9	Sciatic nerve	SC proliferation/trophic signaling	IGFR-1 PDGFR ErbB	(Chattopadhyay and Shubayev, 2009) (Mantuano <i>et al.</i> , 2008) (Kim <i>et al.</i> , 2012)
			SC migration	LRP-1	(Mantuano <i>et al.</i> , 2008)
			SC maturation and myelin ensheathment	Laminin-2	(Kim <i>et al.</i> , 2012), (Gu <i>et al.</i> , 2005) (Court <i>et al.</i> , 2006) (Chernousov <i>et al.</i> , 2008)
	MMP-2/-9	Sciatic nerve/PC12 cells	Neurite regrowth	CSPGs NFM	(Ferguson and Muir, 2000) (Zuo <i>et al.</i> , 1998) (Filous <i>et al.</i> , 2010) (Shubayev and Myers, 2004), (Lariviere and Julien, 2004)
	MMP-2/-9	Sciatic nerve	Macrophage recruitment	TNF precursor Collagen type IV	(Shubayev and Myers, 2000) (Shubayev and Myers, 2002) (Siebert <i>et al.</i> , 2001)
			Regulation of size and composition of SC domains	β -dystroglycan	(Court <i>et al.</i> , 2011)
<i>CNS regeneration</i>	MMP-2	Spinal cord, transplanted OECs and hUCBs	Glial scar breakdown	CSPGs	(Veeravalli <i>et al.</i> , 2009), (Pastrana <i>et al.</i> , 2006) (Hsu <i>et al.</i> , 2006)
		Cochlear nuclear complex	<i>De novo</i> synaptogenesis	Unknown	(Fredrich and Illing, 2010)

	MMP-2	RGCs on OEC monolayers	Axonal regrowth	PNN components	(Pastrana <i>et al.</i> , 2006)
	MMP-9	Spinal cord	Remyelination	NG2	(Larsen <i>et al.</i> , 2003)
		Dentate gyrus	Activity-dependent dendrite remodeling	Unknown	(Szkclarczyk <i>et al.</i> , 2002)
		Barrel cortex	Synaptic reorganization	IGF-1	(Kaliszewska <i>et al.</i> , 2011) (Nishijima <i>et al.</i> , 2010)
		RGC axons	Lesion-induced sprouting of intact neurons	Pro-BDNF	(Penedo <i>et al.</i> , 2009)
	MMP-2/-9	OECs	Glial scar reduction via migration of regeneration promoting cells	Nogo-66 receptor Cytoskeletal components	(Gueye <i>et al.</i> , 2011) (Ogier <i>et al.</i> , 2006)
<i>Adult neurogenesis and angiogenesis</i>	MMP-2	HVC endothelial cells	Neurogenesis/neuronal recruitment/angiogenesis	(Pro-)VEGF	(Kim <i>et al.</i> , 2008)
	MMP-9	Astrocytes and neurons in cerebral cortex	Neurovascular remodeling and functional repair	(Pro-)VEGF	(Zhao <i>et al.</i> , 2006)
	MMP-2/-9	Subventricular zone/dentate gyrus	NPC proliferation, migration and differentiation	Laminin Integrin	(Wang <i>et al.</i> , 2006), (Lee <i>et al.</i> , 2006) (Kang <i>et al.</i> , 2008) (Barkho <i>et al.</i> , 2008) (Wojcik <i>et al.</i> , 2009)
	MMP-2/-9	Human endothelial cells	Angiogenesis	Collagen	(Itoh <i>et al.</i> , 1998) (Nguyen <i>et al.</i> , 2001) (Slevin <i>et al.</i> , 2006)
	MMP-2/-9	Endothelial cells, astrocytes and newborn neurons of dentate gyrus	Neurogenesis and angiogenesis	VEGF Pro-BDNF Pro-NGF	(Lu <i>et al.</i> , 2008)

^a BBB, blood-brain barrier; FGF2, fibroblast-growth factor 2; PKC, protein kinase C; ICAM-5, intercellular adhesion molecule 5; EphB2, ephrin receptor B2; NCAM, neural cell adhesion molecule; DRG cells, dorsal root ganglion cells; NGF, nerve growth factor; ICAM-5, intercellular adhesion molecule-5; NCAM, neural cell adhesion molecule; MBP, myelin basic protein

^b LTP, long-term potentiation; NMDAR, N-methyl-D-aspartate subtype of glutamate receptor; LVDCC, L-type voltage dependent Ca²⁺ channel; DCN, deep cerebellar nuclei; PNN, perineural net; sAAP, secreted form of amyloid precursor protein; CRMP-2, collapsin response mediator protein-2; SynCAM-2, synaptic cell adhesion molecule-2; PNN, perineural net

^c OEC, olfactory ensheathing cell; hUCB, human umbilical cord blood cells; SC, Schwann cell; IGFR-1, insulin growth factor receptor-1; PDGFR, platelet-derived growth factor receptor; LRP-1, LDL-receptor related protein-1; CSPG, chondroitin sulfate proteoglycan; NFM, neurofilament; TNF, tumor necrosis factor; IGF-1, insulin growth factor-1; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; HVC, high vocal center (in birds); NPC, neural precursor cell; RGC, retinal ganglion cell

Figure 1
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Figure 2

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LEGEND

● glutamate

● MMP-9

● MMP-2

● SynCAM-2

● Cav2.2

actin

EphB2

LVDCC

tenascin-c

NMDAR

laminin

CRMP-2

β-dystroglycan

MMP-2/-9 mRNA vesicles

ICAM-5

microtubuli

β1-integrin

Figure 3
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Abbreviations:

MMP, matrix metalloproteinase; ECM, extracellular matrix; TIMP, tissue inhibitor of metalloproteinases; CNS, central nervous system; PNS, peripheral nervous system; SC, Schwann cell; CSPG, chondroitin sulphate proteoglycan; NSC, neural stem cell; aNPC, adult neural progenitor cell; BBB, blood-brain-barrier; LTP, long-term potentiation; NMDAR, N-methyl-D-asparaginezuur receptor; OEC, olfactory ensheathing cell