**Matrix metalloproteinase 9 and epileptogenesis – the crucial role of the enzyme and strategies to prevent the disease development**

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**Abbreviations:** AED – anti-epileptic drug; ECM – extracellular matrix; LTD – long-term potentiation; LTP – long-term depression, MMP-9 – matrix metalloproteinase 9; SE – status epilepticus; TLE – temporal lobe epilepsy

**ABSTRACT**

Epileptogenesis is the process responsible for converting normal brain into an epileptic. It may be triggered by an event such as brain injury or status epilepticus (SE). The main mechanisms responsible include neuroinflammation and blood-brain barrier (BBB) disruption, pathologic neuronal networks’ reorganisation and aberrant synaptic plasticity. Accumulating amount of evidence from animal models and epileptic patients strongly suggest that matrix metalloproteinase 9 (MMP-9) is potentially one of the key executors of the processes of epileptogenesis. MMP-9 by affecting synaptic plasticity is suggested to enable epileptic remodelling of the brain circuitry. MMP-9’s dependent cleavage of BBB followed by inflammatory cell infiltration into the brain contributes to the neuroinflammation component of epileptogenesis. The goal of this review was to analyse all possible ways MMP-9 may be involved in epileptogenesis and consider MMP-9 inhibition as potential therapeutic strategy.

**INTRODUCTION**

Matrix metalloproteinase-9 (MMP-9), a member of the metzincin family of mostly extracellularly operating proteases, has emerged as a critical molecule for brain physiological and pathological processes. Matrix metalloproteinases (MMPs) constitute a large family of extracellular enzymes and together with a disintegrin and metalloproteinases (ADAMs), ADAM proteases with thrombospondin motifs (ADAMTSs) and astacins form the metzincin family of mostly extracellularly operating proteases [1, 2]. Notwithstanding that MMP-9 is just one enzyme of many with similar structures and enzymatic specificities, multiple studies have revealed its unique and prominent role in brain development, synaptic and cortical plasticity as well as its contribution to the pathogenesis of neurodegenerative and psychoneurological disorders, and epilepsy in particular.

Epilepsy is the most common serious neurological condition, with at least 60 million patients worldwide, and almost half a million in Poland. The most widespread form of epilepsy is temporal lobe epilepsy (TLE), characterized by the presence of epileptic foci (usually located within hippocampus, amygdala or temporal neocortex). More than 30% of all epilepsy cases and almost 70% of all TLE cases are resistant to treatments currently available on the market [3-5]. While scientific studies on mechanisms of epilepsy and antiepileptic drugs (AEDs) have a history of more than one century, the focal point of investigations until recently was ictiogenesis (the mechanism of seizure generation in an already developed disease). Thus far, ion channels and neurotransmitter receptors have been on centre stage in epilepsy research, and treatment has conventionally been symptomatic in which “AEDs” treat the symptoms - seizures, but not the underlying disease mechanisms.

Epileptogenesis is the dynamic process responsible for converting normal brain tissue into an epileptic one. It comprises progressive alterations of neuronal excitability, establishment of new critical neuronal interconnections and intricate structural changes, before the first seizure occurs. As recent experimental and data from patients suggest, molecular and cellular changes triggered by an epileptogenic insult can continue to progress after the epilepsy diagnosis, the term epileptogenesis can be extended to also include disease progression. Traumatic brain injury (TBI), tumours, stroke, prolonged symptomatic seizures etc. can induce epileptogenesis and lead to new epileptic foci formation. Epileptogenesis has grown into a separate research field only recently, and despite huge interest in the topic, the exact mechanisms underlying epilepsy progression still remain obscure. Changes taking place in epileptogenic brain encompass neurogenesis and neurodegeneration, damage of blood-brain barrier (BBB) with extensive recruitment of inflammatory cells, gliosis, synaptic plasticity, reorganization both of the extracellular matrix and the molecular architecture of individual neuronal
cells. Clearly comprehending this processes and establishing its key players, we shall be able to develop tools to interfere and prevent the epilepsy advancement. The prevention, delay or seizure modification may be considered as a clinically relevant endpoint for antiepileptogenesis studies [3,6,7].

The objective of this review is to detail various ways, by which MMP-9 is contributing to epileptogenesis, and arrive at holistic view of MMP-9 role in the processes as epilepsy advances, what will help us to construe how potential modifications of its activity may be either beneficial or detrimental for epileptogenic brain. The scientific potential of the issue is confirmed by the fact that this topic has already been covered in comprehensive reviews [8-10].

MMP 9 MOLCULE

MMP-9’S STRUCTURE, EXPRESSION AND ACTIVITY REGULATION

MMP-9, also known as gelatinase B for its ability to cleave gelatine, is a type IV collagenase and 92-kDa protein that belongs to a family of zinc- and calcium-dependent endopeptidases. Structurally MMP-9 is a multidomain enzyme that consists of the following elements: the propetide, removal of which renders MMP-9 active; active site and metal binding site forming the catalytic domain; hemopexin domain (PEX), involved in substrate specificity and interactions with inhibitors and cell surface receptors; three fibronectin (FN) repeats, facilitating the degradation of (large) gelatinous substrates; O-glycosylated (OG) domain, unique to MMP-9 [11]. Generally, MMP-9 operates within the extracellular space, although the intracellular action of MMP-9 has also been detected [2]. MMP-9 was shown to be present within the hippocampus, cerebral cortex, and cerebellum. MMP-9 secretion in an inactive form from dendrites is stimulated by glutamate [11].

MMP-9 gene expression is very low at resting brain, however, it is markedly activated in response to a variety of physiological (e.g., learning) and pathological (e.g., injury) conditions. MMP-9 transcription is influenced by various neurotransmitters, cytokines, chemokines and growth factors. Synergy and antagonism within the extracellular signalling network determine the immunological and physiological outcomes. Kuzniets laska et al. [12] have elucidated a prototypical neuronal MMP-9 gene activation pathway stimulated by Brain Derived Neurotrophic Factor (BDNF) in hippocampal neurons in culture. The pathway involves TrkB BDNF tyrosine kinase receptor driving extracellular signal-regulated kinases to activate serum response factor to stimulate the expression of the c-fos gene and then to trigger MMP-9 gene expression with the aid of AP-1 transcription factor involving c-Fos protein. Other transcriptional regulators, particularly nuclear factor-kB, contribute to MMP-9 expression in both neuronal and glial cells (for an extensive review, see Vandooren et al. [11]). The role of AP-1 in a massive MMP-9 gene activation has been well recognized in non-neuronal systems [13] and also shown to play a major role in MMP-9 gene activation in learning in the brain in vivo – a phenomenon well known to be dependent on glutamate receptors [14]. Seizures also involve those receptors, thus the AP-1 pathway that is highly responsive to seizures [15,16], might be driven by them as well to upregulate MMP-9. However, the seizure-driven MMP-9 gene expression appears to be far more complex, as it also involves epigenetic mechanisms, such as MMP-9 gene promoter demethylation and active role of Polycomb Complex and YY1 transcriptional regulator [17,18]. Of particular importance is the finding that MMP-9 gene promoter demethylation has been demonstrated in human epileptic brain samples and progressive demethylation correlates with epileptogenesis in experimental animals [18].

On the post-transcription level several pathways may activate the degradation of MMP-9 mRNAs [19]. Besides, regulations by microRNAs have been recently reported [20,21]. In neurons an additional level of MMP-9 regulation is its local translation in synapo-dendritic compartment. MMP-9 mRNA is transported to dendrites and locally translated in response to activation stimuli [22,23].

Upon production at the synapse, MMP-9 is released into the extracellular space in an activity-dependent manner in a precursor form. This ProMMP-9 is the subject of activation via a network of enzyme interactions. Another MMPs such as MMP-3, MMP-2, trypsin, plasmin, urokinase-type plasminogen activator (uPA) have been implicated to participate in its activation. Restricted and time-dependent activity of MMP-9 is a result of tight regulation of its expression and activity, which occurs together with co-released MMP-9 endogenous inhibitor TIMP-1 [24]. Notably, the misbalance between MMP-9 and TIMP is associated with numerous CNS diseases [2,8,11]. The possible mechanism involved may be excessive or reduced proteolysis depending on quantity of MMP-9 available at the synapse, or depletion of free TIMP molecules, resulting in reduced TIMP functionality. When unleashed, MMP-9 can cleave its substrates, such as beta-dystroglycan, nectin-3, N-cadherin, integrin beta 1, brain derived neurotrophic factor (BDNF) or nerve growth factor (NGF). MMP-9 abnormal extracellular activity can bring misbalance onto a number of molecular pathways, affecting inflammatory responses, neuron plasticity and cell death, what is described more widely in specific chapters.

MMP-9 ROLE IN SYNAPTIC PLASTICITY

Synaptic plasticity may be defined as activity-dependent re-organization of the synaptic connections that comprises changes both at the morphological and functional level. Recently, the importance of extracellular matrix remodelling by proteases has been reconsidered to be essential for synaptic plasticity [25]. Among extracellularly operating proteases MMP-9 is deemed to be the main candidate responsible for synapse remodelling. MMP-9 specific postsynaptic localization and local dendritic/synaptic translation additionally support the fact of the enzyme important role in the plasticity of excitatory synapses. Wang et al. [26] reported MMP-9 availability as a prerequisite for the enlargement of spines associated with long-term potentiation (LTP) induction – the most widely used model of synaptic plasticity (see Włodarczyk et al. [27], for review). MMP-9 was suggested to perform an instructive role in establishing persistent modifications in both synapse structure and function [26]. Excitatory synapses responsible for plasticity are located on
dendritic spines – exactly where MMP-9 has been reported to be released and activated. Michaluk et al. [28] have reported that enzymatic activity of MMP-9 causes elongation and thinning of dendritic spines in hippocampal neurons in three independent experimental models. This finding was in accordance with Wilczynski et al. [4] findings, who comparing the spines’ size with their MMP-9 level, showed that thinner and smaller spines contain more MMP-9. Additionally, Michaluk and colleagues showed that the process was mediated through integrin beta 1, whose blockage abolished the effect on spine morphology caused by MMP-9 [28]. Furthermore, MMP-9 potentiated NMDA receptors mobility along the dendritic cell membrane and this effect was also integrin beta 1 dependent [29]. On top of that, MMP-9 and NMDA receptors are known to mutually activate each other [30]. On the other hand, acting through the integrins, MMP-9 can recruit GluA1 AMPA receptor [31-33]. MMP-9 substrate – ICAM-5 – can affect the expression of AMPA receptors within the synapse [34]. Most researchers are equivocal that either MMP-9 or MMP-9 released products can stimulate spine elongation. MMP-9 inhibition, in contrast, promotes the transformation of dendritic spines toward mature, mushroom shaped spines [24,35,36].

MMP-9 PATHOPHYSIOLOGY IN EPILEPTOGENESIS

EVIDENCE FROM EXPERIMENTAL EPILEPTOGENESIS MODELS

Back in 1994 Rosenberg showed that 92 kD metalloproteinase, induced by haemorrhagic injury, took part in a proteolytic cascade that broke down extracellular matrix, opening the blood-brain barrier with secondary brain oedema and cell death [37]. It is widely known that level of MMPs is increased during neuropsychological diseases that are accompanied by an inflammatory component, so, reasonably, the starting point of investigations were bound to MMP-9s aggravation of immune response and its epileptogenic consequences [38]. On other hand, an alternative approach on MMP-9 role in epileptic brain has also been proposed based on unique MMP-9 functions at the synapse leading to epilepsy circuitry establishment [6]. In the following chapters will be reviewing scientific evidence dealing with different aspects of MMP-9 engagement into epileptogenesis.

The link between MMP-9 and seizures was initially reported by Zhang and colleagues who identified MMP-9, along with another gelatinease - MMP-2, in the different regions of the seizing rat brain, with the highest activity in hippocampus [5]. Notably, hippocampus is the region known to be particularly prone to brain insults and undergo significant plasticity-related structural changes in epileptogenesis. The seizures were evoked by kainate administration, which not only induces severe status epilepticus, but also leads to neurodegeneration in all hippocampal subfields except for the dentate gyrus (DG), where ingrowth of mossy fibres (granule cell axons) into their parent dendritic field to make recurrent excitatory connections is observed [4]. Bearing typical histopathological reminiscent of human TLE, kainate epileptogenesis model is considered as one of the most reliable and is widely adopted. Thus, Zhang et al. [3] demonstrated the correlation between expression of MMP-9 and neuronal activity during convulsions, showing that MMP-9 was rapidly induced in brain regions prone to kainate administration. MMP-2, on the other hand, was upregulated with some delay and its activity was bound to neurodegeneration. The fact that bicusculine (able to induce seizures without neurodegeneration observed) upregulated MMP-9 but not MMP-2, additionally supported this notion [5].

Szklarczyk et al. [1] investigated the topic further and showed MMP-9 strong expression by neurons and to some degree by glial cells, while MMP-2 was found to be expressed predominantly by astrocytes. Kainate treatment resulted in upregulation of MMP-9 mRNA, protein, and enzymatic activity with unique spatiotemporal characteristics, in particular in the hippocampal dentate gyrus. MMP-9 activation correlated specifically with neuronal remodeling and not cell loss. Substantial evidence of MMP-9 link to seizure development have been obtained by Wilczynski et al. [4] in pentylenetetrazole (PTZ) kindling – induced epilepsy model. By using MMP-9 genetically modified animals a direct link between metalloproteinase and epileptogenesis was established. MMP-9 knockout mice demonstrated less pronounced epileptic seizures, while rats with MMP-9 overexpression an increased tendency to epileptogenesis and increased seizure severity [4]. Recently, Pijet et al. [39] has demonstrated MMP-9 upregulation in controlled cortical impact (CCI) mice epilepsy model. MMP-9 overexpression increased the number of mice that exhibited TBI-induced spontaneous seizures, and MMP-9 knockout decreased the appearance of seizures. All these data confirm the strong link between MMP-9 upregulation and seizures, and urge us to invest our attention into the topic and learn about exact mechanisms of MMP-9 action epilepsy pathogenesis.

NEUROINFLAMRATION AND BBB DISRUPTION

Epilepsy-triggering initial insult induces a highly regulated cascade of biological events, accompanied by a release of cytokines, chemokines, lipid mediators, and protectins in the neuronal microenvironment. It is regarded that unregulated inflammatory processes lead to aberrant neural connectivity and the hyper-excitable neuronal network, which mediate the onset of epilepsy [11,40]. Thus we start with multifaceted characterization of MMP-9 in epileptogenesis by inspecting its role in neuroinflammation, as the this may be considered as a starting point for the whole avalanche of deleterious events resulting in epilepsy.

MMPs are known to play an outstanding role in immune cell development, ligand-receptor interactions, effector function and cell migration, and MMP-9 certainly is not an exception. MMP-9, in particular, was reported to activate proinflammatory cytokines namely tumour necrosis factor alpha (TNF-α), IL-8, granulocyte chemotactic protein, etc. Moreover, MMP-9 promotes the chemotactic signal by releasing the chemokines from ECM. During the formation of a new epileptic foci microglia are being activated and astrocytes turn reactive. MMP-9 takes part in activation and deactivation of many proinflammatory chemokines and cytokines produced by these cells (IL-1β, IL-6, TNF-α, and TGF-β) [11,41]. Interestingly, not only can MMP-9 intensify, but also inhibit chemotaxis [42,43]. Thus, its double role in the regulation of
pro- and anti-inflammatory processes has been recognized. When it comes to epileptic process, it was observed that factors that are considered to be proinflammatory do not always deepen damage, but also may exert protective function during status epilepticus. MMP-9 may be to a certain extent responsible for the maintenance of homeostasis between protection and damage, which needs further elucidation before planning to intrude in natural pattern of MMP-9 activity in the brain [16].

Still, taking into account MMP-9 modulation of inflammatory pathways, the most prominent input of this metalloproteinase to epileptogenesis is made by influencing the BBB integrity and aftereffects that follow. It is known that SE-induced BBB disruption plays an important role in the development of epilepsy and progression of seizure activity [44]. It can impact the development of epilepsy directly (via influx of potassium) or as a result of glial activation, impaired potassium buffering, inflammation, and synaptogenesis triggered by serum proteins which penetrate the brain via leaky BBB [45]. Studies in animal models suggest, that BBB leakage can be detected within minutes after SE induction, be extensive during the latent period and not abate completely until the chronic epileptic phase is reached [8].

Considering the pathological contribution of BBB disintegration in the progress of epilepsy, the fact that MMP-9 is a main factor participating in blood-brain barrier damage independently of damaging factor, is of major importance. This destructive influence mainly stems from the fact that MMP-9 can cleave molecules responsible for BBB tightness. Zonula occludens 1 protein, responsible for tight junction regulations, collagen type IV, a main component of the basal lamina of endothelium have been proven among MMP-9 substrates [46]. Arguably, MMP-9 can on top of that hew occludin, a building block of tight junction connections [47].

Increased brain MMP-9 levels render BBB leak, which promotes brain infiltration by leukocytes. MMP-9 is largely responsible for transmigration of T cells to the site of action [40]. Invading T cells secrete additional amounts of MMP-9, thus maintaining this feedback loop. Another mechanism for MMP-9 to contribute to BBB disruption is the metalloproteinase involvement in the release of cytokines and free radicals. MMP-9 knockout mice have been reported resistant in models, where condition is acquired due to impaired BBB [26].

ABNORMAL MMP-9 PROTEOLYTIC ACTIVITY IN EXTRACELLULAR SPACE

As the essential MMP-9 milieu is extracellular space, it is instinctive to anticipate that MMP-9 dependent cleavage of the extracellular matrix mediates the changes leading to epileptogenesis. ECM is not a rigid and passive structure, its components play an important role in maintaining ion homeostasis, modulating activity of ion channels and receptors. Some researchers consider ECM to be the fourth component of the synapse (together with pre- and postsynaptic part of neuron and astrocyte component, “tetra-partite synapse”, see Dityatev et al. [48]. Perineuronal nets – big clusters of the ECM that surrounds subpopulations of neurons recently has been reported to perform neuroprotective functions [8,49]. MMP-9 dependent cleavage of trans-synaptic cell adhesion molecules can influence trans-synaptic interactions leading to pathological conditions. Beta-dystroglycan and nectin-3 have been proven as MMP-9 substrates [50,51].

Dystroglycan is a component of dystrophin-glycoprotein complex. Its function is to links the cytoskeleton to the extracellular matrix and stabilize the dystrophin-glycoprotein complex at the membrane. beta-Dystroglycan downregulation was correlated with abnormal epileptiform activation of neurons in vitro. Moreover, this phenomena was shown to be associated with the dysfunctions of astrocytic end feet, implying the BBB malfunction [52]. Increase in MMP-9 dependent nectin-3 cleavage in hippocampus, on the other hand, was shown to be the cause of memory impairment and cognition and therefore, is speculated to be partly responsible for epilepsy-comorbid cognitive decline [51]. Another trans-synaptic adhesion protein neurelin-1 can be cleaved by MMP-9[53]. Neuroligins play an important role in the formation of synaptic contacts and balancing the number of excitatory and inhibitory neuronal contacts, which imbalance is established as epilepsy cause.

ABERRANT NEUROGENESIS AND NEURON PROLIFERATION

In the response to brain injury, the mobilization of endogenous neural stem cells, in order to substitute lost neurons, causes the increase in the number of newly generated neurons [54]. Considerable fraction of these newly born cells were shown to migrate into the granule cell layer and develop morphological and electrophysiological features of mature dentate granule cells [55]. Their abnormal migration and proliferation potentially contributes to the formation of epileptogenic hippocampal circuitry observed after acute seizures or brain injury. Unexpectedly, hippocampal neurogenesis was also reported to be reduced at chronic stages of epilepsy [56] and virtually complete lack of adult brain neurogenesis in cyclin D2 knockout mice did not affect epileptogenesis [57]. Nevertheless, it has been hypothesized that suppression of abnormal hippocampal neurogenesis may be a strategy to mitigate seizure intensity. Keeping in mind all abovementioned facts, MMP-9 was suggested to be involved in mechanisms that mediate neurogenesis and cell migration [58]. In adult monkey MMP-9 was shown to be upregulated in acute and delayed phases of the post ischemic reaction and co-localised with markers of new-born neurons in the subgranual zone. Moreover, MMP-9 co-localization with new born neurons from the subventrical zone during the recovery period after transient focal cerebral ischemia was reported [59].

POSSIBLE ROLE IN CELL DEATH

MMP-9 contribution to cell death is still an issue of controversy. MMP-9 was repeatedly reported to cause cell death by impairing the transmission between ECM and the cell via the lipoprotein receptor-related protein and by separation of the cells from ECM leading to anoikis [60,61]. Lee et al. [42] observed gelatinase-dependent initiation of caspase-mediated cytotoxicity in brain endothelial cells, with MMP inhibitors significantly decreasing caspase-3 activation and reducing endothelial cell death. It should be mentioned that
inhibitor applied was of a broad range of MMP-9 specificity, so the effect cannot be assigned to MMP-9 alone.

When it comes to data obtained in epilepsy models, evidence of MMP-9 contribution to cell death are more ambiguous. In pilocarpine model of epilepsy Kim and colleagues reported that MMP-9 induced apoptotic hippocampal cell death by interrupting integrin-mediated survival signalling after SE. Again, treatment with MMP-9 inhibitor showed neuroprotective effect [29]. Further evidence to exclude MMP-9 influence on cell death come from Wilczynski, who showed that MMP-9 inhibitor had no effect on neuronal damage in organotypic slice culture treated with kainate [4]. Earlier, Szkłarczyk et al. [9] showed MMP-9 to be strongly expressed by neurons and to a lesser degree by glial cells, whereas MMP-2 was expressed predominantly by astrocytes. Induction in GFAP correlated with increased MMP-2 expression both spatially and temporally, which suggest, that astrocytes utilize this enzyme to modify their surrounding ECM, facilitating migration toward reactive brain regions and promoting neuritic regeneration.

**PATHOLOGICAL SYNAPTIC PLASTICITY IN EPILEPTOGENESIS**

Aberrant synaptic plasticity is believed to be a pathogenic factor in neurodegenerative and neuropsychiatric conditions [62]. In epileptogenesis neuronal circuits undergo reorganization of various neurotransmitter/neuromodulator systems, with the balance shifting toward excitation, eventually yielding in seizures. Crucially, MMP-9 modifies both NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) ionotropic receptors, the central mediators of excitatory neurotransmission [11]. Level of expression of these receptors has been shown to be altered during different phases of epileptogenesis. Seizures are reported to stimulate integrin and matrix protein expression, together with extracellular proteolysis. This facilitates reactive axonal growth and circuit modification following seizures.

Considering the interplay between MMP-9 on the one side, and NMDA and AMPA receptors on the other, MMP-9 importance in long-term potentiation and depression (LTP and LTD, respectively) can be inferred. Memory impairment is common debilitating comorbidity to epilepsy, and LTP and LTD paradigms are the most widely studied physiological models of mammalian memory formation [63]. Essentially, LTP mechanisms of memory are similar to those underlying epileptogenesis by kindling: both LTP and kindling are most effectively evoked by high frequency stimuli, involve synaptic facilitation and share overlapping molecular mechanisms.

In epileptogenesis, changes at DG dendritic tree following initial insult can be divided dependent on time course, into two phases: the early spine pruning and subsequent chronic progressive (aberrant) synaptogenesis. MMP-9 KO and wildtype mice have been compared for the degree of acute spine loss in kainic acid model of epilepsy. In wildtype animals a substantial decrease in the density of spines occurred unilaterally to the injection, while in knockouts no such an effect took place [4]. Hence, lack of MMP-9 render mice more resistant to epileptic damage. MMP-9 KO mice has also been reported not to undergo such plastic phenomenon in the dentate gyrus of hippocampus, as pruning of dendritic spines and axonal sprouting that involves ingrowth of mossy fibres into their parent dendritic field to make recurrent excitatory connections [64]. These changes are observed not only in chemically-induced animal models of epilepsy, but also are typical for human TLE. The sprouting is speculated to underlie spontaneous seizures. Aberrant synaptogenesis on the contrary, that occurs days to weeks after initial insult, is not associated with spine pruning, but rather with an increase in spine number. Seizure-related changes in spines are believed to represent a mechanistic basis for cognitive deficits in epilepsy [6].

**EVIDENCE FROM CLINICAL STUDIES**

It shall be stressed that direct knowledge transfer from animal models to human conditions is not simple. In fact, the discrepancies in the mechanisms of disease progression between the animal model and the disease in human itself may lead to choosing the wrong drug candidate, leading to failure in clinical trials. Luckily, in contrast to number of other diseases, where only post-mortem samples are available, analysis of human brain tissue excised during the neurosurgical operations in patients with drug resistant epilepsy may be performed. Studies on brain surgery tissue found increases in MMP-9 immunoreactivity in epileptogenic lesions associated with focal cortical dysplasia and tuberous sclerosis and in epileptogenic cortical or hippocampal lesions in patients with temporal lobe epilepsy without underlying cytoarchitectonic abnormalities [65–67]. Interestingly, an increase of MMP-9 activity was also reported in the unchanged tissue of patients with epilepsy. The increase of MMP-9 level was mostly marked postsynaptically, within the dendritic spines. Moreover, the MMP-9 level in blood of epileptic patients was found to be increased [65]. The increase of the MMP-9 level and the MMP-9 to TIMP-1 ratio was observed in patients with acute encephalopathy after febrile seizures, what was associated with blood-brain barrier damage [68]. Although in patients who developed epilepsy as a complication of acute encephalitis no discrepancies of MMP-9 levels were found, the MMP-9 to TIMP-1 ratio was significantly increased [69]. Moreover, the mean MMP-9 to TIMP-1 ratio was higher in patients with worse control of epilepsy (defined by authors as a minimum of 28 seizures during four weeks) than in the patients in whom lower number of seizures was observed [8]. Similarly, the level of MMP-9 and MMP-9 to TIMP-1 ratio in blood serum was increased in children with influenza caused encephalopathy, in the group who developed febrile seizures [69]. In patients with TLE qualified for lobectomy, serum MMP-9 levels upon operation has decreased. In these patients the level of MMP-9 proenzyme and activity in the tissue of epileptic focus was increased in comparison to surrounding tissues and a normal hippocampus [67]. The study performed on with generalized tonic-clonic seizures, patients with convulsive and non-convulsive status epilepticus, and patients with complex partial seizures significant increase of serum MMP-9 in 1 hour and in 24 hours after seizures has been observed, with the return to control level in 72 hours after seizures [70]. In another study cerebrospinal fluid level
of MMP-9 in patients with bacterial meningitis who subsequently developed epileptic seizures was higher than in group with no seizure developed [71].

MMP-9 INHIBITION AS ANTIETEPILEPTOGENIC THERAPEUTIC STRATEGY

If we are to move from proof-of-principle studies to preclinical, and even clinical testing, a number of challenges should be dealt with. First, looking retrospectively onto the vast amount of data obtained in rodent models of epileptogenesis, one can notice significant differences in the pattern of molecular changes, time course and severity of the cellular alterations depending on a model, such as electrically or chemically induced SE or TBI, not to mention inter-animal variability. Therefore, reproducibility of results among different animal models or even species should be confirmed first, and patients target group (post-TBI or post-SE acquired epilepsy) carefully selected. Secondly, as acquired epileptogenesis was shown to be regulated by multiple molecular pathways, it would be prudent to avoid oversimplification that the modulation of a single target pathway can be antiepileptogenic [3].

However, MMP-9 seems to overtake these obstacles successfully. Its involvement in epileptogenesis has been proved in different models of epileptogenesis, both chemically (kainic acid, PTZ kindling, bicuculine, pilocarpine) and mechanically (TBI) induced in mice and rats, including knockouts and MMP-9 overexpressing animals. Even more importantly, multiple reports from clinical studies show increased MMP-9 in patients having seizures of different etiologies. As revised above, MMP-9 is proven to contribute multilaterally to fundamental processes of epileptogenesis, which makes it an attractive drug target to pursue.

Because of high structural homology of the active sites of MMPs, the design of potent and selective inhibitor is a demanding task. Lack of selectivity may result in severe side effects, the main reason of clinical trials failure of MMP-9 inhibitors developed against cancer. Moreover, with a few exceptions, most of MMP-9 inhibitors developed so far lack an ability to penetrate BBB, a prerequisite for an antiepileptogenic candidate. Still, some attempts in this way are being made. Yeghiazaryan et al. [72] has reported the compound DP-b99 to delay onset and severity of PTZ-induced seizures in mice, display neuroprotective effect on kainate excitotoxicity and block MMP-9 dependant morphological reorganization of the dendritic spines. Recently, novel peptidomimetic MMP-9 inhibitors have been shown to penetrate BBB and be effective in epileptogenesis [73].

Another issue for scrutiny is the fact that MMP-9 role in epileptogenesis is rather dual than negative, and at some points MMP-9 is speculated to be neuroprotective and important in maintaining homeostasis [8]. As MMP-9 is released in specific time and space pattern, further investigation, showing at what time points MMP-9 activity is damaging and must be inhibited, and when not, would be necessary. To enable matching drug delivery pattern, ad-
vanced controlled-release formulations must be developed, which is a challenge in terms of pharmacokinetics. Witnessing the stall in CNS drug discovery and ineffectiveness of conventional drug design approaches, novel MMP-9 inhibitors having cutting edge formulation and unique pharmacodynamic and pharmacokinetic properties, may be the first therapeutics of the new era.

CONCLUSIONS

Epileptogenesis prevention is one of the most important and unmet challenges for the pharmaceutical industry, and a huge amount of evidence hint MMP-9 as a perfect drug target to investigate. Its physiological role in the processes of neuroinflammation, cell differentiation, tissue remodeling, angiogenesis, regulation of the level and activity of cytokines and growth factors, synaptic plasticity has been proven. Abnormal MMP-9 activity has been regarded to contribute to the formation of epileptic foci, pathological synaptic connections and neuronal networks. Diverse roles of MMP-9 in epileptogenesis may be summarized in Figure 1. Clinical studies strongly confirm the link between MMP-9 and seizure development. Still, proceeding from proof-of-concept studies to successful drug candidate development is a daunting challenge, and additional research attempts to explicate the MMP-9s role in epileptogenesis are to be undertaken.

REFERENCES

1. Szklarczyk A, Lapinska J, Rylski M, McKay RDG, Kaczmarek L (2002) Matrix metalloproteinase-9 undergoes expression and activation during diencephalic remodeling in adult hippocampus. J Neuroscience 22: 920-930
2. Vafadari B, Salamian A, Kaczmarek L (2016) MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J Neurochem 139 Suppl 2: 91-114
3. Pitkanen A, Lukasiuk K (2011) Mechanisms of epileptogenesis and potential treatment targets. Lancer Neurol 10: 173-186
4. Wilczynski GM, Konopacki FA, Wilczek E, Lasiecia Z, Gorlewicz A, Michaluk P, Jaworski J, Kaczmarek L, Koperski L, Duniec K, Mioduszewska B, Nikolaev E, Walczak A, Owczarek D, Gorecki DC, Zuschratter W, Ottersen OP, Kaczmarek L (2008) Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol 180: 1021-1035
5. Zhang JW, Deb S, Gottschall PE (1998) Regional and differential expression of gelatinases in rat brain after systemic kainic acid or bicuculline administration. Eur J Neurosci 10: 3358-3368
6. Lukawski K, Andres-Mach M, Czuczwar M, Luszczki JJ, Kriszynski K, Czuczwar SJ (2018) Mechanisms of epileptogenesis and preclinical approach to antiepileptic therapies. Pharmacol Rep 70: 284-293
7. Pitkanen A, Engel J Jr (2014) Past and present definitions of epileptogenesis and its biomarkers. Neurotherapeutics 11: 231-241
8. Bronisz E, Kurkowska-Jastrzebska I (2016) Matrix metalloproteinase 9 in epilepsy: the role of neuroinflammation in seizure development. Mediators Inflamm 2016: 7369020
9. Lukasiuk K, Wilczynski GM, Kaczmarek L (2011) Extracellular proteins in epilepsy. Epilepsy Res 96: 191-206
10. Yin P, Yang L, Zhou HY, Sun RP (2011) Matrix metalloproteinase-9 may be a potential therapeutic target in epilepsy. Med Hypotheses 76: 184-186
11. Vandooren J, Van den Steen PE, Opdenakker G (2013) Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. Crit Rev Biochem Mol Biol 48: 222-272
12. Kuzniarska B, Rejnak E, Malik AR, Jaworski J, Kaczmarek L, Kalita K (2013) Brain-derived neurotrophic factor induces matrix metalloproteinase 9 expression in neurons via the serum response factor/c-Fos pathway. Mol Cell Biol 33: 2149-2162
13. Kaczmarek L, Lapinska-Dzwoniec J, Szymczak S (2002) Matrix metalloproteinases in the adultbrain physiology: a link between c-Fos, AP-1 and remodeling of neuronal connections? EMBO J 21: 6643-6648
14. Ganguly K, Rejnak E, Mikosz M, Nikolaev E, Knapaska E, Kaczmarek L (2013) Matrix metalloproteinase (MMP) 9 transcription in mouse brain induced by fear learning. J Biol Chem 288: 20978-20991
15. Kaminska B, Filipkowski RK, Zurkowska G, Lason W, Przewlocki R, Kaczmarek L (1994) Dynamic changes in the composition of the AP-1 transcription factor DNA-binding activity in rat brain following kainite-induced seizures and cell death. Eur J Neurosci 6: 1558-1566
16. Lukasiuk K, Savonenko A, Nikolaev E, Rydz M, Kaczmarek L (1999) Defensive conditioning-related increase in AP-1 transcription factor in the rat cortex. Brain Res Mol Brain Res 67: 64-73
17. Rylski M, Amborska R, Zybura K, Mioduszewska B, Michaluk P, Jaworski J, Kaczmarek L (2008) Yin Yang 1 is a critical repressor of matrix metalloproteinase-9 expression in brain neurons. J Biol Chem 283: 35140-35153
18. Zybura-Broda K, Amborska R, Ambrosek-Latecka M, Wilemska J, Bogus A, Bucko J, Konopka A, Grajankowska W, Roszkowski M, Marchel A, Rysz A, Kopierski L, Wilczynski GM, Kaczmarek L, Rylski M (2016) Epigenetics of epileptogenesis-evoked upregulation of matrix metalloproteinase-9 in hippocampus. PLoS One 11: e0157945:1-25
19. Zybura-Broda K, Wolder-Gontarek M, Ambrosek-Latecka M, Choros A, Bogus A, Wilemska-Dzidziszyczka J, Rylski M (2018) HuB (Elavl1) and HuB (Elavl2) stabilize matrix metalloproteinase-9 mRNA during seizure-induced Mmp-9 expression in neurons. Front Neurosci 12: 224
20. Jasinska M, Milek J, Cynerman IA, Leski S, Kaczmarek L, Dziembowska M (2016) miR-132 Regulates dendritic spine structure by direct targeting of matrix metalloproteinase-9 mRNA. Mol Neurobiol 53: 4701-4712
21. Konopka W, Kiryk A, Novak M, Herwther M, Parkitna JR, Wawrzynia- ak M, Kowarsch A, Michaluk P, Dzwonek J, Armsterger T, Wilczynski G, Merkenschlager M, Theiss FJ, Kofir G, Kaczmarek L, Schütz G (2010) MicroRNA loss enhances learning and memory in mice. J Neurosci 30: 14835-14842
22. Dziembowska M, Milek J, Janusz A, Rejnak E, Romanowska E, Gorkiewicz T, Tiron A, Bramham CR, Kaczmarek L (2012) Activity-dependent local translation of matrix metalloproteinase-9. J Neurosci 32: 14538-14547
23. Janusz A, Milek J, Perycz M, Pacini L, Bagni C, Kaczmarek L, Dziembowska M (2013) The Fragile X mental retardation protein regulates matrix metalloproteinase 9 mRNA at synapses. J Neurosci 33: 18234-18241
24. Magnowska M, Gorkiewicz T, Suska A, Wawrzyniak M, Rutowska-Wlodarczyk I, Kaczmarek L, Wlodarczyk J (2016) Transient ECM protease activity promotes synaptic plasticity. Sci Rep 6: 27757
25. Tsililary E, Tzinia A, Radenovic L, Stamenkovic V, Lebtko T Mucha M, Pawlak R, Frischknecht R, Kaczmarek L (2014) Neural ECM proteases in synaptic plasticity. Front Neurosci 8: 285
26. Wang XB, Bozdagi O, Nikitczuk JS, Zhai ZW, Zhou Q, Huntley GW (2017) Extracellular proteolysis by metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. Proc Natl Acad Sci USA 105: 19520-19525
27. Wlodarczyk J, Mukhina I, Kaczmarek L, Dityatev A (2011) Extracel- lular matrix molecules, their receptors, and secreted proteases in synap- tic plasticity. Dev Neurobiol 71: 1040-1053
28. Michaluk P, Wawrzyniak M, Alet P, Szczot M, Wrayembek P, Mercik K, Medvedev N, Wilczek E, De Roo M, Zuschratter W, Muller D, Wilczynski GM, Mozyczmas JW, Stewart MJ, Kaczmarek L, Wlodarczyk J (2017) Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. J Cell Sci 124: 3369-3379
29. Michaluk P, Mikosz B, Groc L, Frischknecht R, Choquet D, Kacz- marek L (2009) Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin beta1 signaling. J Neurosci 29: 6007-6012
48. Dityatev A, Rusakov DA (2011) Molecular signals of plasticity at the tetrapartite synapse. Curr Opin Neurobiol 21: 353-359
49. Ferrer-Ferrer M, Dityatev A (2018) Shaping synapses by the neural extracellular matrix. Front Neuroanat 12: 40
50. Michaluk P, Kołodziej L, Mioduszewska B, Wilczynski GM, Dziewonk J, Jaworski J, Gorecki DC, Ottersen OP, Kaczmarek L (2007) Beta-dystroglycan as a target for MMP-9, in response to enhanced neuronal activity. J Biol Chem 282: 16136-16141
51. van der Kooij MA, Fantin M, Rejmak E, Grosse J, Zanoletti O, Fournier C, Ganguly K, Kalita K, Kaczmarek L, Sandi C (2014) Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. Nat Commun 5: 4995
52. Gondo A, Shinotsuka T, Morita A, Abe Y, Yasui M, Nuriya M (2014) Sustained down-regulation of beta-dystroglycan and associated dysfunctions of astrocytic endfeet in epileptic cerebral cortex. J Biol Chem 289: 30279-30288
53. Peixoto RT, Kunz PA, Kwon H, Mabb AM, Sabatini BL, Philpot BD, Ehlers MD (2012) Transsynaptic signaling by activity-dependent cleavage of neuregulin-1. Neuro 76: 396-409
54. Zhong Q, Ren BX, Tang FR (2016) Neurogenesis in the hippocampus of patients with temporal lobe epilepsy. Curr Neurol Neurosci Rep 16: 20
55. Ramirez-Amaya V, Marrone DF, Gage FH, Worley PF, Barnes CA (2006) Integration of new neurons into functional neural networks. J Neurosci 26: 12237-12241
56. Danzer SC (2016) Neurogenesis in epilepsy: better to burn out or fade away? Epilepsy Curr 16: 268-269
57. Kondratuk I, Plucinska G, Miszczuk D, Wozniak G, Szydlowska K, Kaczmarek L, Filipkowski RK, Lukasik K (2015) Epileptogenesis following kainic acid-induced status epilepticus in cyclin d2 knock-out mice with diminished adult neurogenesis. PLoS One 10: e0128285:1-9
58. Wojcik-Stanaszek L, Gregor A, Zalewska T (2011) Regulation of neurogenesis by extracellular matrix and integrins. Acta Neuropathol (Wars) 71: 103-112
59. Lu L, Tonchev AB, Kaplambadzhiev DB, Boneva NB, Mori Y, Sahara S, Ma D, Nakaya MA, Kikuchi M, Yamashima T (2008) Expression of matrix metalloproteinases in the neurogenic niche of the adult monkey hippocampus after ischemia. Hippocampus 18: 1074-1084
60. Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, Lipton SA (2002) S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science 297: 1186-1190
61. Wang X, Lee SR, Ariai K, Lee SR, Tsuji K, Rebeck GW, Lo EH (2003) Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med 9: 1313-1317
62. Dziewonska M, Wiloarczyk J (2012) MMP-9: a novel function in synaptic plasticity. Int J Biochem Cell Biol 44: 1039-1046
63. Ferrari-Bergomi S, Spagnoli G, Satta S, Domenici G, Wiśniewski R, Mordorski M, Gubaszewski W, Fratelli M, Tonon RC, Lipton SA (2002) S-nitrosylation of matrix metalloproteinases: a new pathway to neuronal cell death. Science 297: 1186-1190
64. Wang X, Lee SR, Ariai K, Lee SR, Tsuji K, Rebeck GW, Lo EH (2003) Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med 9: 1313-1317
65. Ferrari-Bergomi S, Spagnoli G, Satta S, Domenici G, Wiśniewski R, Mordorski M, Gubaszewski W, Fratelli M, Tonon RC, Lipton SA (2002) S-nitrosylation of matrix metalloproteinases: a new pathway to neuronal cell death. Science 297: 1186-1190
66. Li S, Yu S, Zhang C, Shu H, Liu S, An N, Yang M, Yin Q, Yang H (2012) Increased expression of matrix metalloproteinase 9 in cortical lesions from patients with focal cortical dysplasia type IIb and tuberous sclerosis complex. Brain Res 1453: 46-55
67. Quirico-Santos T, Nascimento M, Cacimiro Gomes A, de Carvalho LP, de Souza JM, Alves-Leon S (2013) Increased metalloproteinase activity in the epileptogenic lesion – Lobectomy reduces metalloproteinase activity and urokinase-type uPAR circulating levels. Brain Res 1538: 172-181
68. Suenaga N, Ichiyama T, Kubota M, Isumi H, Tohyama J, Furukawa S (2008) Roles of matrix metalloproteinase-9 and tissue inhibitors of metal-
Metaloproteinaza macierzy pozakomórkowej 9 i epileptogeneza – kluczowa rola enzymu i strategie zmierzające do zapobiegania rozwojowi choroby

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STRESZCZENIE

Epileptogeneza jest procesem odpowiedzialnym za przemianę zdrowego mózgu w padaczkowy. Ten proces może być spowodowany przez takie zdarzenia, jak uraz mózgu czy stan padaczkowy. Głównym mechanizmem odpowiedzialnym za zmiany mogą być, przede wszystkim, stan zapalny mózgu, zakłócenia ciągłości bariery krew-mózg, patologiczna przebudowa sieci neuronalnych oraz nienaturalna plastyczność synaptyczna. Dane eksperymentalne uzyskane w modelach zwierzęcych oraz u pacjentów z padaczką sugerują, że metaloproteinaza macierzy zewnątrzkomórkowej 9 (MMP-9) jest potencjalnie jednym z kluczowych składników procesu epileptogenezy. MMP-9 wpływa na plastyczność synaptyczną umożliwiając padaczkową reorganizację obwodów neuronalnych w mózgu. Zależne od MMP-9 cięcie elementów bariery krew-mózg i następująca po nim infiltracja komórek układu immunologicznego do mózgu, przyczyniają się do stanu zapalnego w epileptogenezie. Celem danej pracy przeglądowej jest analiza możliwych mechanizmów zaangażowania MMP-9 w proces epileptogenezy oraz koncepcja zahamowania MMP-9 jako potencjalnej strategii terapeutycznej.