



## Degrading Epilepsy: The Role of Extracellular Proteases and the Extracellular Matrix

### Matrix Metalloproteinase-9 Contributes to Kindled Seizure Development in Pentylentetrazole-Treated Mice by Converting Pro-BDNF to Mature BDNF in the Hippocampus.

Mizoguchi H, Nakade J, Tachibana M, Ibi D, Someya E, Koike H, Kamei H, Nabeshima T, Itohara S, Takuma K, Sawada M, Sato J, Yamada K. *J Neurosci* 2011;31:12963–12971.

Recurrent seizure activity has been shown to induce a variety of permanent structural changes in the brain. Matrix metalloproteinases (MMPs) function to promote neuronal plasticity, primarily through cleavage of extracellular matrix proteins. Here, we investigated the role of MMP-9 in the development of pentylentetrazole (PTZ)-induced kindled seizure in mice. Repeated treatment with PTZ (40 mg/kg) produced kindled seizure, which was accompanied by enhanced MMP-9 activity and expression in the hippocampus. No change in MMP-9 activity was observed in the hippocampi of mice with generalized tonic seizure following single administration of PTZ (60 mg/kg). MMP-9 colocalized with the neuronal marker NeuN and the glial marker GFAP in the dentate gyrus of the kindled mouse hippocampus. Coadministration of diazepam or MK-801 with PTZ inhibited the development of kindling and the increased MMP-9 levels in the hippocampus. Marked suppression of kindled seizure progression in response to repeated PTZ treatment was observed in MMP-9<sup>-/-</sup> mice compared with wild-type mice, an observation that was accompanied by decreased hippocampal levels of mature brain-derived neurotrophic factor. Microinjecting the BDNF scavenger TrkB-Fc into the right ventricle before each PTZ treatment significantly suppressed the development of kindling in wild-type mice, whereas no effect was observed in MMP-9<sup>-/-</sup> mice. On the other hand, bilateral injections of pro-BDNF into the hippocampal dentate gyrus significantly enhanced kindling in wild-type mice but not MMP-9<sup>-/-</sup> mice. These findings suggest that MMP-9 is involved in the progression of behavioral phenotypes in kindled mice because of conversion of pro-BDNF to mature BDNF in the hippocampus.

### Commentary

The extracellular matrix (ECM) consists of a complex, mesh-like network of acellular structures and molecules that surround and envelop neurons, glia, and synapses in the brain. Although the cellular elements of the brain are obviously critical for epileptogenesis and seizure generation, the ECM is increasingly recognized as also playing an important role in the pathophysiology of neurologic diseases, including epilepsy (1). The ECM may potentially influence epileptogenesis in a number of ways, mostly related to structural plasticity within the brain. Extracellular structural networks may normally stabilize cellular and synaptic elements and prevent anatomic plasticity under physiologic conditions. In pathologic states, however, these extracellular components may break down and allow abnormal structural reorganization, such as axonal sprouting or dendritic spine loss, which may promote epileptogenesis and other neurologic complications of epilepsy.

The specific mechanisms by which changes in the ECM trigger epileptogenic processes in the brain are poorly understood. Recently, a number of extracellular proteases have been identified that may mediate both physiologic and pathologic effects of the ECM via degradation of extracellular structural elements and signaling molecules (2). For example, matrix metalloproteinases (MMPs) represent a family of zinc-dependent secreted or cell membrane-bound enzymes involved in remodeling of the ECM (3, 4). Some MMPs are activated intracellularly before being transported to the membrane or secreted, whereas other MMPs are first secreted as an inactive proenzyme into the extracellular space and then converted into an activated form. MMPs can cleave structural proteins and signaling molecules in the ECM to regulate both physiologic and pathologic processes. One type of MMP, MMP-9, regulates dendritic spine morphology and synaptic plasticity related to learning mechanisms, such as long-term potentiation (5). MMP-9 also has been implicated in the pathophysiology of a number of neurologic disorders (3, 4).

The potential role of MMP-9 in epileptogenesis is supported by several lines of evidence. MMP-9 levels are elevated in the serum of children with prolonged febrile seizures and status epilepticus (6) as well as in the brains of several animal



models of epilepsy (7–9). Furthermore, MMP-9 knock-out mice exhibit a decreased progression of epileptogenesis in a chemical kindling model, which is correlated with an inhibition of seizure-evoked pruning of dendritic spines and a decrease in synaptogenesis after mossy fiber sprouting (9).

The molecular targets of MMP-9 that promote epileptogenesis are currently not known. Empirically, a rational mechanism could involve MMP-mediated cleavage of ECM structural proteins, such as collagen, laminin, and proteoglycans, which could secondarily affect morphologic changes associated with epileptogenesis, such as axonal sprouting and dendritic spine loss. MMP-9 also may regulate cell death and apoptosis occurring during epileptogenesis in some cases. Alternatively, MMP-9 may induce proteolytic activation of other enzymes or signaling molecules, which could then have a number of downstream structural or biochemical effects via various extracellular or intracellular signaling pathways.

The recent study by Mizoguchi et al. supports previous work implicating MMP-9 in epileptogenesis and provides initial evidence for a molecular mechanism by which MMP-9 may regulate epileptogenic processes. A chemical kindling model in mice is utilized, in which repetitive application of initially subconvulsant doses of the drug pentylenetetrazole (PTZ) eventually leads to a decreased seizure threshold and increased seizure severity. They first confirm previous findings that MMP-9 is elevated during epileptogenesis and that MMP-9 knock-out mice have delayed kindling (9). The most novel aspect of the study suggests that MMP-9 affects epileptogenesis via conversion of pro–brain-derived neurotrophic factor (BDNF) to the active form of BDNF, because BDNF levels were increased by PTZ kindling and because inhibiting or increasing BDNF had corresponding inhibitory or excitatory effects on the development of PTZ kindling in wild-type mice only but not in MMP-9 knock-out mice. Like MMP-9, BDNF is also a secreted peptide and can regulate extracellular networks related to synaptic plasticity as well as intracellular processes via membrane receptors and gene regulation. Because BDNF itself has also been implicated in epileptogenesis (10), the apparent activation of BDNF by MMP-9 provides a potential mechanistic link to explain the actions of MMP-9 in epilepsy.

Like most interesting studies, this paper raises a number of unanswered issues that need to be addressed. Although the MMPs are believed to act primarily via degradation of structural proteins of the ECM, PTZ kindling surprisingly caused no changes in the levels of several standard MMP substrates, such as laminin and beta-dystroglycan. Thus, it remains to be determined whether MMP-9 truly influences epileptogenesis via structural changes in the ECM or rather exerts its effects via downstream biochemical or functional processes. In this regard, PTZ kindling did significantly increase BDNF levels; these levels were partially blocked in the MMP-9 knock-out mice, suggesting that BDNF may be the initial downstream mediator of MMP-9 actions. However, whereas BDNF levels were increased at multiple time points during PTZ kindling in wild-type mice, BDNF levels were only inhibited in the MMP-9 knock-out mice during the early stages of PTZ kindling. Thus, the limited time window during which MMP-9 appears to activate BDNF raises questions about the specificity and mechanisms of this interaction during epileptogenesis. Furthermore,

even if BDNF activation is the immediate target of MMP-9, because BDNF may have multiple cellular and molecular effects on synaptic plasticity, cell survival, and excitability (10), the downstream effectors of MMP-9/BDNF activation remain unknown. In theory, these could involve both structural and functional/biochemical changes in either the extracellular or intracellular compartments.

Independent of the downstream mechanisms involved, the potential role of MMP-9 in epileptogenesis has direct therapeutic implications. Pharmacologic inhibitors of MMPs exist and represent feasible candidates for antiepileptogenic therapies. Given the extracellular localization of activated MMPs, targeting MMP-9 may constitute a novel therapeutic mechanism with fewer adverse effects than more traditional treatment for seizures that primarily inhibit neuronal excitability. Furthermore, intrinsic biological MMP inhibitors, named tissue inhibitors of matrix metalloproteinases (TIMPs), coexist with MMPs and likely function to rapidly inhibit the action of MMPs, thus limiting the duration of MMP action and preventing excessive tissue degradation and injury. Potentiation of TIMPs may represent a complementary therapeutic strategy to MMP inhibitors. Despite the therapeutic potential for modulating MMPs, there are currently little data testing the effect of MMP inhibition on epilepsy in animal models or people. However, the existing work in preclinical models suggests that regulation of MMPs and the ECM may represent a rational treatment for epilepsy.

by Michael Wong, MD, PhD

## References

1. Dityatev A. Remodeling of extracellular matrix and epileptogenesis. *Epilepsia* 2010; 51(suppl):61–65.
2. Lukasiuk K, Wilczynski GM, Kaczmarek L. Extracellular proteases in epilepsy. *Epilepsy Res* 2011;96:191–206.
3. Rosenberg GA. Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. *Lancet Neurol* 2009;8:206–216.
4. Mizoguchi H, Yamada K, Nabeshima T. Matrix metalloproteinases contribute to neuronal dysfunction in animal models of drug dependence, Alzheimer's disease, and epilepsy. *Biochem Res Int* 2011;2011:1–10.
5. Wang XB, Bozdagi O, Nikitczuk JS, Zhai ZW, Zhou Q, Huntley GW. Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. *Proc Natl Acad Sci USA* 2008;105:19520–19525.
6. Suenaga N, Ichiyama T, Kubota M, Isumi H, Tohyama J, Furukawa S. Roles of matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 in acute encephalopathy following prolonged febrile seizures. *J Neurol Sci* 2006;226:126–130.
7. Jourquin J, Tremblay E, Decanis N, Charton G, Hanessian S, Chollet AM, Le Diquardher T, Khrestchatsky M, Rivera S. Neuronal activity-dependent increase of net matrix metalloproteinase activity is associated with MMP-9 neurotoxicity after kainate. *Eur J Neurosci* 2003;18:1507–1517.
8. Takacs E, Nyilas R, Szepesi Z, Baracszy P, Karlens B, Rosvold T, Bjorkum AA, Czurko A, Kovacs Z, Kekesi AK, Juhasz G. Matrix metalloproteinase-9 activity increased by two different types of epileptic seizures that do not induce neuronal death: A possible role in homeostatic synaptic plasticity. *Neurochem Int* 2010;56:799–809.



9. Wilczynski GM, Konopacki FA, Wilczek E, Lasiecka Z, Gorlewicz A, Michaluk P, Wawrzyniak M, Malinowska M, Okulski P, Kolodziej LR, Konopka W, Duniec K, Mioduszevska B, Nikolaev E, Walczak A, Owczarek D, Gorecki DC, Zuschratter W, Ottersen OP, Kaczmarek L.

Important role of matrix metalloproteinase 9 in epileptogenesis. *J Cell Biol* 2008;180:1021–1035.  
10. Binder DK. The role of BDNF in epilepsy and other diseases of the mature nervous system. *Adv Exp Med Biol* 2004;548:34–56.



## PAME CONFERENCE

June 21-24, 2012  
Hilton Orrington/Evanston  
Chicago, IL

### You Are Invited to...

the first **Partners Against Mortality in Epilepsy (PAME)** conference devoted predominantly to Sudden Unexpected Death In Epilepsy (SUDEP) where clinical, basic science and patient/family attendees will come together to understand and support each other.

## CALL FOR ABSTRACTS

*(50 will be accepted)*

*Please pass this invitation on to your patients, and their support networks!*

**June 21-24, 2012 ■ Chicago, IL**

AES is proud to partner with CDC, CURE, EFA, ETP/FACES, NINDS, and SUDEP Aware to bring this first of its kind, 3-day learning event to our community.

**Only 300 spaces available, so register soon!**

**More Information: <http://www.aesnet.org/pame/>**



# American Epilepsy Society

## Epilepsy Currents Journal

### Disclosure of Potential Conflicts of Interest

#### Section #1 Identifying Information

1. Today's Date: 3/11/11

2. First Name Michael Last Name Wong Degree MD, PhD

3. Are you the Main Assigned Author?  Yes  No

If no, enter your name as co-author \_\_\_\_\_

Manuscript/Article Title: Degrading Epilepsy: The Role of Extracellular Proteases and the

Extracellular Matrix

4. Journal Issue you are submitting for: 12.3

#### Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship just add rows to this table.

Type	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**
1. Grant	x				
2. Consulting fee or honorarium	x				
3. Support for travel to meetings for the study or other purposes	x				
4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like	x				
5. Payment for writing or reviewing the manuscript	x				
6. Provision of writing assistance, medicines, equipment, or administrative support.	x				
7. Other	x				

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

**Section #3 Relevant financial activities outside the submitted work.**

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship just add rows to this table.

Type of relationship (in alphabetical order)	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**
1. Board membership	x				
2. Consultancy	x				
3. Employment	x				
4. Expert testimony	x				
5. Grants/grants pending			Yes	NIH, Citizens United for Research in Epilepsy, McDonnell Center, Washington University/Pfizer collaboration	Pre-clinical research grant; no clinical studies.
6. Payment for lectures including service on speakers bureaus	x				
7. Payment for manuscript preparation.	x				
8. Patents (planned, pending or issued)	x				
9. Royalties	x				
10. Payment for development of educational presentations	x				
11. Stock/stock options	x				
12. Travel/accommodations/meeting expenses unrelated to activities listed.**	x				
13. Other (err on the side of full disclosure)	x				

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

**Section #4 Other relationships**

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

  x   No other relationships/conditions/circumstances that present a potential conflict of interest.

     Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.  
*Epilepsy Currents* Editorial Board