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The (Extracellular) Matrix Reloaded: Imaging Matrix Metalloproteinase Activity

Gelatinase Biosensor Reports Cellular Remodeling During Epileptogenesis

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Epileptogenesis is the gradual process responsible for converting a healthy brain into an epileptic brain. This process can be triggered by a wide range of factors, including brain injury or tumors, infections, and status epilepticus. Epileptogenesis results in aberrant synaptic plasticity, neuroinflammation, and seizure-induced cell death. As matrix metalloproteinases (MMPs) play a crucial role in cellular plasticity by remodeling the extracellular matrix, gelatinases (MMP-2 and MMP-9) were recently highlighted as key players in epileptogenesis. In this work, we engineered a biosensor to report in situ gelatinase activity in a model of epileptogenesis. This biosensor encompasses a gelatinase-sensitive activatable cell penetrating peptide (ACPP) coupled to a TAMRA fluorophore, allowing fluorescence uptake in cells displaying endogenous gelatinase activities. In a preclinical mouse model of temporal lobe epilepsy, the intrahippocampal kainate injection, ACPPs revealed a localized distribution of gelatinase activities, refining temporal cellular changes during epileptogenesis. The activity was found particularly but not only in the ipsilateral hippocampus, starting from the CA1 area and spreading to dentate gyrus from the early stages throughout chronic epilepsy, notably in neurons and microglial cells. Thus, our work shows that ACPPs are suitable molecular imaging probes for detecting the spatiotemporal pattern of gelatinase activity during epileptogenesis, suggesting their possible use as vectors to target cellular reactive changes with treatment for epileptogenesis.

Commentary

Acquired epilepsies (AE) are those resulting from a known precipitating brain insult. From a scientific perspective, AEs are particularly convenient to study (compared to idiopathic or cryptogenetic epilepsies) as the epileptogenic period has a clearly defined beginning. Animal models of AE include post-traumatic epilepsy (PTE) involving mechanical trauma to the brain and post-status epilepticus (SE) models in which epilepsy develops after acute induction of SE. Both forms of AE can grossly be divided into 3 phases: acute injury, latent period, and chronic epilepsy.

The injury itself involves acute neuronal death, vascular damage, and/or axonal shearing (in PTE models). The chronic phase is characterized by spontaneous recurrent seizures, which may lead to ongoing cell death and circuit reorganization that worsen the epileptic condition. The latent phase is arguably the most interesting/useful phase of AE to study as it represents the time period during which epilepsy has yet to be established and prophylactic therapeutic intervention may be feasible. There is a substantial catalog of data describing the anatomical and pathophysiological changes that occur throughout the latent phase.[1] These changes include secondary progression of injury such as hypoxia–ischemia-induced neuronal and glial death resulting from vascular damage during the primary injury and excitotoxicity resulting from acute seizures during the primary injury. The changes also include presumed "restorative" processes such as neurogenesis, gliogenesis, axonal and dendritic sprouting, synaptogenesis, and angiogenesis. While a lot is known about these *cellular* changes in brain circuitry that occur during the latent period, very little is known about the *extracellular* remodeling that is also taking place.

The extracellular "space" in which brain cells reside is actually not just space, but rather a sophisticated gelatinous matrix of proteoglycans and link proteins that is secreted by cells and comprises approximately 20% of the brain.[2] During development, the spatiotemporal evolution of the extracellular matrix (ECM) guides neurogenesis, migration, and axon growth. In the mature brain, the ECM mediates synaptic plasticity and prevents aberrant synaptic remodeling.[3] In this way, the basic element of neural transmission could more accurately be described as tetra—rather than tripartite: involving pre- and postsynaptic neurons, glia, and the ECM. Recent evidence further suggests that highly variable sulfation of the ECM creates a variable density of negative charge, which in turn alters the local concentration of extracellular chloride (and GABA reversal potential) via the Gibbs-Donnan effect.[4]

The ECM's role in synaptic stabilization and neuronal excitability place it in a unique position to determine brain rhythms. Neurons remodel the ECM using an array of gelatinases called matrix metalloproteinases (MMPs). Interestingly, MMP9 in particular has been implicated in epileptogenesis based on assays of tissue resected from humans and multiple animal models of epilepsy.[5,6] However, tools for monitoring ECM remodeling in situ are lacking and thus so is specific information about the role of the ECM and MMPs in epileptogenesis.

In the highlighted study, a novel tool for monitoring gelatinase activity is presented.[7] This biosensor is an activatable cell-penetrating peptide (ACPP): A fluorophore-labeled cell-penetrating polycation connected in a hairpin configuration via a gelatinase-sensitive linker to a neutralizing polyanion. When the linker is cleaved by gelatinase (specifically MMP2 or MMP9), the cation enters nearby cells and labels them with the red fluorophore to which it is fused. In this way, cells releasing MMPs are the most likely to be labeled with the fluorophore. After validating the biosensor *in vitro*, the authors sought to identify a population of cells that actively remodel the ECM at different phases of epileptogenesis. Epilepsy was induced in mice using the well-characterized post-SE model of AE: unilateral intrahippocampal injection of kainic acid (KA). Mice were sacrificed at 1 of 3 time points: 24 hours post-KA, 1 week post-KA, or 8 weeks post-KA. One week before sacrificing the animals, ACPP was injected into both hippocampi. The hippocampus ipsilateral to the KA injection site showed elevated ACPP staining at all 3 time points sampled. The contralateral hippocampus exhibited widespread, low, but significant ACPP staining at 24 hours and 7 days but more localized staining at 8 weeks. Sham saline injections produced low levels of ipsilateral ACPP staining and no contralateral staining. Staining of laminin (a key, gelatinase-sensitive component of the ECM) revealed a negative image to the ACPP staining, further validating that ACPP is identifying active gelatinase digestion of the ECM. Together these results support the hypothesis that MMP activity is significantly elevated in AE.

A key advantage of ACPP-based labeling is that it identifies specific cells releasing MMPs. To begin to catalog cells responsible for ECM remodeling in the intrahippocampal kainate model of AE, the authors co-stained the harvested tissue with labels for neurons, astrocytes, and microglia. Pyramidal cells of CA1 were strongly labeled at 24 hours and 7 days and sparsely labeled at 8 weeks post-KA. Astrocytes were transiently labeled at 7 days, but not at 8 weeks post-KA, while microglia exhibited low-level ACPP staining at all 3 time points. Sham animals only exhibited low-level microglial staining at the injection site. Together, these results point to neurons as the primary source of MMPs throughout epileptogenesis. The persistent microglial staining is somewhat difficult to interpret, as microglia may phagocytose dying cells that contain the TAMRA fluorophore and thus ACPP staining may not indicate MMP activity in the microglia themselves.

As ACPP is an extracellular stain that translocates to the nearest cells upon cleavage by MMP, its specificity to labeling the cell that released the MMP cannot be guaranteed. However, images in the highlighted paper show clear high-contrast labeling of individual neurons, with nearly no labeling of neighboring cells in some cases. This suggests not only that ACPP specifically labels cells releasing the MMPs but also proposes an interesting model of AE, wherein a subset of cells play a more prominent role in remodeling the ECM. Another apparent limitation of ACPP is that 1 week after administration, the dye appears to be primarily compartmentalized in the soma, effectively identifying the cells releasing MMPs, but obscuring *where* the MMPs were acting on the ECM. Despite the spatial and temporal shortcomings of ACPP, the highlighted study represents a novel look at the processes underlying ECM remodeling during AE and identifies ACPP as a useful addition to the somewhat lacking toolset for studying the ECM. It would be interesting to extend this study to look for common patterns of MMP activity in animal models of PTE, which more directly model human epilepsy. Furthermore, since ACPPs do not require genetic alterations to the species being studied, they could be applied to study PTE in large animals,[8] giving insight into the ECM remodeling that takes place following trauma in a gyrencephalic brain.

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