Journal Club

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# PERK-STING Signaling Drives Neuroinflammation in Traumatic Brain Injury

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Review of Sen et al.

Traumatic brain injury (TBI) resulting from excessive contact in sports, blast injuries in war, or occupational hazards is a widespread public health concern. Symptoms associated with TBI include neurocognitive deficits, psychiatric disorders, and encephalopathies. Neuroinflammation plays an integral role in the pathophysiology of TBI. Although neuroinflammation is initially beneficial, because it promotes debris clearance and regeneration, prolonged neuroinflammation elicits secondary injury, leading to progressive neurodegeneration (Simon et al., 2017). Dysregulated neuroinflammation also contributes to neurodegenerative diseases, brain cancers, and neurological infections (Dickens et al., 2017; Bright et al., 2019; Doron et al., 2019). Therefore, targeted immunomodulation is an actively pursued therapeutic avenue for treating these conditions. Such strategies aim to restrict acute inflammation to protective levels, prevent chronic neuroinflammation, and maximize regenerative programs.

Previous work has identified several contributors to secondary injury related to inflammation after TBI. Karve et al. (2016) found that type I interferons (IFNs) such as IFN$\beta$ are upregulated in postmortem human TBI brains and that IFN$\beta$ activates proinflammatory microglia in mouse models of TBI. Sen et al. (2017) showed that phosphorylation of protein kinase R-like endoplasmic reticulum kinase (PERK) initiates endoplasmic reticulum (ER) stress after TBI, and PERK blockade rescued synaptic damage and memory deficits. Liu et al. (2012) showed that ER stress involves activation of the ER protein stimulator of interferon genes (STING), and Abdullah et al. (2018) found that STING expression was elevated in postmortem human TBI brains. Abdullah et al. (2018) also showed that STING deletion suppressed TBI-induced type I IFNs, reduced lesion volume, and attenuated microglial reactivity in mouse models. Notably, microglia, the key regulators of neuroinflammation, can be classically activated to adopt a proinflammatory (M1) phenotype or alternatively activated to adopt an anti-inflammatory (M2) phenotype. These studies establish that TBI polarizes microglia toward an M1 phenotype and that ablation of STING-induced type I IFNs produces an M2-polarizing environment.

Sen et al. (2020) sought to establish a link between PERK and STING in the pathophysiology of TBI. They found that subjecting adult mice to unilateral controlled cortical impact, a TBI paradigm, led to robust phosphorylation of PERK, STING, and interferon regulatory factor 3 (IRF3; a downstream STING target that initiates transcription of type I IFNs) in neurons. Administration of a PERK inhibitor or a PERK siRNA abrogated TBI-induced STING phosphorylation, IRF3 phosphorylation, and IFN$\beta$ expression in pericontusional cortices, suggesting that PERK activates STING during TBI. Consistent with this, treating primary neuronal cultures with the ER stress inducer tunicamycin increased STING phosphorylation, IRF3 phosphorylation, and IFN$\beta$ expression. Tunicamycin had no effect on primary microglia cultures. However, treating microglia with conditioned media from tunicamycin-treated primary neurons, which was rich in IFN$\beta$, induced activation and polarization to an M1 phenotype (evidenced by phosphorylation of the proinflammatory transcription factor NF-kB), and treatment with a PERK inhibitor attenuated M1 polarization. Finally, in the TBI model, a PERK inhibitor reduced multiple pathological features that occur in response to neuroinflammation driven by M1 microglia, including T-cell infiltration, loss of white matter proteins, thinning of the corpus callosum, loss of oligodendrocytes, and increased anxiety- and depressive-like behaviors.

The identification of PERK-STING signaling by Sen et al. (2020) is an important advance in our understanding of neuroinflammatory mechanisms with

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potentially wide-ranging implications for immunology. STING is canonically activated by cyclic GMP-AMP (cGAMP) generated by the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), which detects foreign pathogenic DNA and mislocalized genomic or mitochondrial DNA (Barber, 2015; Chin, 2019; Motwani et al., 2019). STING can also be activated noncanonically, independently of cGAS and cGAMP, by various triggers including double-strand DNA breaks (Dunphy et al., 2018), membrane perturbation (Holm et al., 2012), and infection with *Brucella abortus* (Costa Franco et al., 2018), *Streptococcus pyogenes* (Movert et al., 2018), or influenza A virus (Holm et al., 2016). Noncanonical STING activation by double-strand DNA breaks results in preferential activation of NF-kB over IRF3 (both are needed for expressing IFN$\beta$), requires ubiquitination, and lacks characteristics of canonical STING activation such as STING phosphorylation and translocation to perinuclear foci (Dunphy et al., 2018). Holm et al. (2012) observed that PI3K signaling may be involved in nucleic acid-independent, or cGAS-independent, STING activation. Noncanonical STING activation by infection occurs via various mechanisms, including bacterially-derived cyclic dinucleotides (Costa Franco et al., 2018), virulence factors (Movert et al., 2018), and hemagglutinins (Holm et al., 2016). Despite observing STING phosphorylation, a feature of canonical STING activation, several findings by Sen et al. (2020) support a cGAS-independent mechanism of STING activation by PERK in neurons during TBI. Although the authors did not measure cytosolic DNA or cGAMP levels to definitively exclude the involvement of cGAS, they found that STING was activated selectively in neurons and not glial cells; because cGAS is enriched in microglia and astrocytes, but not in neurons (Cox et al., 2015; Reinert et al., 2016), these findings suggest noncanonical activation of STING in neurons. In addition, it was previously shown that administering cGAMP does not lead to phosphorylation of PERK and other ER stress transducers, demonstrating that cGAS signaling and ER stress are likely decoupled (Imanishi et al., 2019). Ascertaining whether STING is canonically or noncanonically activated during TBI may pave the way for identifying the molecular mechanisms by which PERK activates STING.

A study by Barrett et al. (2020) published recently in the *Journal of Neuroscience* sheds additional light on STING activation following TBI. Notably, Barrett et al. (2020) found acute (within 72 h post-injury) upregulation of cGAS and STING expression in a mouse model of TBI. However, during the chronic phase of TBI (60 d post-injury), only STING expression and not cGAS expression remained upregulated. Because Barrett et al. (2020) neither assessed cytosolic DNA or cGAMP levels nor inhibited cGAS, it is unclear whether cGAS activation causes neuroinflammation during TBI and whether STING switches mechanisms of activation over time.

Further elucidation of PERK-STING signaling, particularly how noncanonical activation might affect downstream mechanisms, is crucial for developing STING-targeting strategies to treat TBI. STING agonists and antagonists are currently being developed to treat various diseases such as lupus, interferonopathy, and cancer, and they show promising results in clinical trials (Sheridan, 2019). The FDA-approved antiviral nucleoside analog ganciclovir was shown to suppress microglial reactivity and neuroinflammation in a STING-dependent manner, but the exact mechanism of action is unknown (Mathur et al., 2017). Significant drug discovery efforts have focused on developing cyclic dinucleotides that directly target the cGAMP binding site on STING (Berger et al., 2019), but it is unlikely these ligands would be effective for cGAS-independent STING signaling. Pharmacological inhibition of noncanonical STING activation would presumably necessitate targeting allosteric sites because cGAMP binding, by definition, does not play any role. Recently, a noncyclic dinucleotide small-molecule inhibitor was developed that functions by covalently modifying the STING transmembrane domain (Haag et al., 2018). Small-molecule PERK inhibitors, including those similar to the compound used by Sen et al. (2020), are being actively developed for neurological diseases and cancer (Cubillos-Ruiz et al., 2017; Halliday et al., 2017), but progress has been hampered by significant pancreatic toxicity because of excessive type I IFN signaling (Yu et al., 2015). STING inhibitors, which have already been extensively administered in clinical trials (Sheridan, 2019), can circumvent this complication and may be a more attractive therapeutic avenue for TBI.

Although Sen et al. (2020) showed that PERK activates STING, they did not inhibit or knock down STING to explicitly demonstrate that the observed increases in IRF3 phosphorylation and IFN$\beta$ production were STING-dependent. This is notable because literature on the link between ER stress and IRF3 activation is stimulus- and context-dependent. Liu et al. (2012) demonstrated in mouse embryonic fibroblasts that tunicamycin induces STING-independent IRF3 phosphorylation. Contrastingly, Sen et al. (2020) observed that tunicamycin induced STING and IRF3 in neurons. Additionally, the observation by Sen et al. (2020) that tunicamycin-induced ER stress in microglia failed to recapitulate the phenotypic effects of IFN$\beta$ treatment, combined with the cell type-specific involvement of STING in IRF3 activation (Liu et al., 2012; Sen et al., 2020) and the significant enrichment of STING in microglia over neurons in C57BL/6 mice (Reinert et al., 2016; the background used by Sen et al., 2020), suggests that there may be neuron-specific coupling of tunicamycin-induced ER stress and IRF3 activation.

Together, the findings by Sen et al. (2020) present a novel and pharmacologically targetable signaling axis underlying neuroinflammation during secondary injury after TBI that not only harbors clinical potential but also spurs a myriad of interesting basic science questions in neuroscience and immunology.

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