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ABSTRACT: Recently, we described a role for the circadian clock protein and nuclear receptor Rev-erbα in regulating glial activation states in the brain. Deletion of Rev-erbα resulted in microglial as well as astrocytic activation, while a Rev-erbα agonist diminished the severity of lipopolysaccharide (LPS)-induced neuroinflammation. Concomitant with this glial activation is impaired neuronal health. These findings suggest that Rev-erb proteins may play critical roles in glial biology. Pertinent ideas such as the glial cell type of most importance, the translatability of these findings to human disease, and the effect of manipulating Rev-erbs in models of neurodegeneration, need to be explored further. In this commentary, we will address the potential role of Rev-erbs in neuroinflammation related to neurodegenerative diseases and speculate on Rev-erbs as potential therapeutic targets for these conditions.

KEYWORDS: Circadian, Rev-erb alpha, microglia, neuroinflammation

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In a recent article,[1] our group described a role for the circadian protein Rev-erbα in regulating glial activation. We demonstrate time-of-day oscillations in microglial morphology/activation that are abrogated by the global deletion of Rev-erbα, which appears to lock microglia in an activated state. Transcriptomic changes in the hippocampus of Rev-erbα KO (RKO) mice also showed upregulation in inflammatory gene expression and activation of pathways associated with inflammation, including the NF-κB pathway. As Rev-erbα is a dedicated transcriptional repressor, we performed ChIP analyses and found that Rev-erbα bound to the promoter regions of NF-kB-related genes, including *TRAF2* and *IKBA*, and may directly regulate NF-kB activation. In vivo, astrocytes are also activated in RKO mice. However, whereas RKO primary microglia are activated at baseline in vitro, RKO primary astrocytes are not. These data suggest a cell-autonomous role for Rev-erbα in microglial activation, and a cell non-autonomous effect on astrocytes. RKO-mediated neuroinflammation resulted in impairments to neuronal health in vitro and on a global brain network level. Using the Rev-erb agonist SR9009, we showed that the Rev-erb pathway can be targeted to mitigate lipopolysaccharide (LPS)-induced neuroinflammation in vitro and in vivo. While this article presents all the detrimental effects of deleting Rev-erbα, we explored a more nuanced view on the results in this commentary.

Rev-erbα has previously been shown to regulate peripheral innate immunity.[2] Macrophages play a critical role in peripheral innate immunity. Given the role of Rev-erbα in macrophages, we explored how Rev-erbα could affect microglial function/activation. Classically, macrophage and microglial activation have been classified using the M1/M2 dichotomy. The M1 state is associated with neurotoxicity, whereas the M2 is associated with neurotrophic effects.[3] Recent advances in transcriptomic analyses have challenged this classification. Our RKO microglia do not clearly fit into either of these categories. On one hand, we noted increases in the classical NF-κB signaling cascade that would suggest an M1 state. However, our microarray and CD68 staining data would suggest increases in phagocytic capacity, which suggests an M2 state. This further illustrates the idea that microglial activation exists on a spectrum and is not so easily classified dichotomously. Rev-erbα deletion may induce a hybrid activation state that needs to be explored in future studies employing microglial-specific transcriptomics. Recent work also suggested that activated microglia can induce neurotoxic A1 astrocyte activation.[4] Our data indicate that astrocyte activation in RKO mice does not fully recapitulate the gene expression changes of an A1 or A2 astrocyte activation state, although most pan-reactive transcripts are increased. Rev-erbα functions as a nuclear receptor and repressor that recruits HDAC3 through NCoR.[5] The loss of this repressive element in the epigenome may prime glia to be more reactive to innocuous stimuli, resulting in spontaneous glial activation and neuroinflammation.

In addition to their homeostatic functions, microglia have recently gained attention for their potential causative role in neurodegenerative pathogenesis. Recent genome-wide association studies indicated that mutations in microglial and phagocytosis genes are highly associated with increased Alzheimer disease (AD) risk.[6] One of those genes is *TREM2*, which we found to be upregulated in the hippocampus of RKO mice. Of note, *TREM2* is a damage-associated microglial gene which is upregulated in microglia surrounding amyloid plaques.[7] Deletion of *TREM2* is known to impair

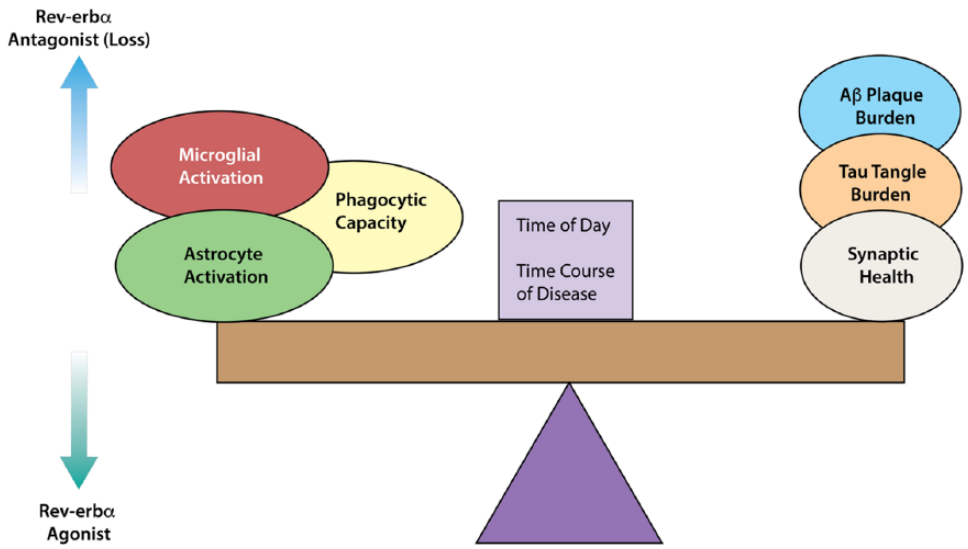

Figure 1. Considerations for the targeting of Rev-erbs in neurodegenerative diseases. Hypothetical model showing the balance between modulating glial activation and altering various aspects of neurodegenerative disease. As noted, activation of Rev-erbs could limit glial activation, which might exert protective effects of synaptic health, but could exacerbate Aβ and tau accumulation. The effects of Rev-erb may be modulated by circadian influence, as well as stage of disease.

microglial responses to amyloid plaques and disrupt microglial synapse elimination.

Rev-erbα deletion is also associated with increased microglial phagocytic activation—as seen by increased CD68/Iba1 colocalization in RKO microglia. Other work has also shown that increases in microglial CD68 can result in increased synaptic pruning in the hippocampus.[8] These suggest that Rev-erbα in microglia can potentially regulate synaptic elimination, perhaps through regulation of *TREM2*. Although Aβ plaque and Tau tangle formation are the classical neuropathological hallmarks of AD, decline in synaptic health has been found to be most closely correlated with cognitive decline.[9] Thus, deeper understanding of the processes that affect synaptic health is critical. Given the increases in phagocytic capacity of RKO microglia, it is reasonable to hypothesize a decline in synaptic health in AD models lacking Rev-erbα. However, the effects may not be so straightforward. Increases in microglial phagocytosis can result in reduced Aβ burden in the brain,[10] but can also exacerbate Tau pathology.[11] To fully understand the role of Rev-erbα in disease, further studies are necessary. However, we speculate that drug interventions through Rev-erbα could influence neurodegenerative pathogenesis through modulation of glial function.

Rev-erbα is a heme/lithium sensitive nuclear receptor that functions as part of the circadian clock molecular machinery.[12] Given this, Rev-erb activity can be modulated using existing agonists and antagonists, including SR9009[13] and SR8278.[14] We used SR9009 in vivo and in vitro and found that it was effective in mitigating LPS-induced neuroinflammation. SR9009 has favorable pharmacokinetics and can readily cross the blood brain barrier. However, the potency of SR9009 is relatively low, as micromolar quantities are required in vitro to exert effects, thus opening the door to off-target effects. With regard to suppressing LPS-induced inflammation, we observed that the efficacy of SR9009 was greatly reduced in RKO mice, suggesting that SR9009 is indeed acting through Rev-erbα in this setting. Our data, as well as data from other groups,[15] provide proof of principle that pharmacologic targeting Rev-erbα can mitigate LPS-induced neuroinflammation and support the development of high-affinity agents.

Although results from our group and others[15] are encouraging, there is a considerable knowledge gap that must be addressed before attempting to predict the effect of Rev-erb targeted drugs in neurodegenerative diseases. First, LPS-induced neuroinflammation is very different than the inflammation observed in neurodegenerative diseases. As an example, *TREM2* is suppressed by LPS, but is upregulated by amyloid plaque pathology. Thus, the effects of Rev-erbα manipulation will need to be tested in neurodegenerative models, as the effects on specific pathways may be quite different. Second, simply reducing neuroinflammation may not be enough to ameliorate neurodegenerative sequelae, as glial activation can exert differing effects on various aspects of pathology at different points in disease course. Using AD as an example, inhibiting neuroinflammation through Rev-erbα activation could abolish the early protective effects provided by increased phagocytic capacity, as more phagocytic microglia could clear plaques and tangles more efficiently and delay the onset of neurodegeneration. Conversely, Rev-erbα activation could provide protection from synapse loss later in the disease course. In addition, continuous activation/blockade of Rev-erbα could be detrimental since our data show that microglial activation cycles by time of day, and these cycles may be important for normal brain homeostasis. Finally, aged microglia are primed and exhibit

decreased phagocytosis and exaggerated responses to stimuli. Therefore, targeting microglial Rev-erbα in aged animals or people would further compromise their ability to function as phagocytes. This suggests that we would need to consider 2 axes of time when considering using Rev-erbα—time of day and time course of the disease. To fully leverage Rev-erbα as a therapeutic target in neurodegenerative disease, all of the outlined factors have to be considered. A summary of the ideas illustrated is in Figure 1.

Although this commentary begins to consider the potential implications of targeting Rev-erbα in neurodegenerative disease, more in vivo studies are required. On a more fundamental level, future studies need to determine the cell type specificity of Rev-erbα in mediating neuroinflammation. Our studies were done in global RKO mice and could have contributing effects from peripheral immune cells or neuronal damage. Future studies need to parse out the effects of knocking out Rev-erbα in only microglia or astrocytes. Regarding the role of Rev-erb in neurodegeneration, studies are needed to examine the effect of Rev-erbα manipulation, either genetic or pharmacologic, in mouse models that address multiple aspects of pathology. Finally, the impact of these interventions on cellular circadian clocks and sleep cycles will need to be addressed. Although this area of investigation is still in its infancy, Rev-erb-targeted drugs may present a novel avenue for optimizing glial responses in neurodegenerative and other neurological disease.

Author Contributions

PG, JMD, and ESM conceived and wrote the manuscript. PG created the figure.

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