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Relationship Status Update on Astrocytic VEGFR-3 and mTOR Signaling: It's Complicated

Vascular Endothelial Growth Factor Receptor-3 Regulates Astroglial Glutamate Transporter-1 Expression via mTOR Activation in Reactive Astrocytes Following Pilocarpine-Induced Status Epilepticus

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Recent evidence has shown that the vascular endothelial growth factor (VEGF) system plays a crucial role in several neuropathological processes. We previously reported an upregulation of VEGF-C and its receptor, VEGFR-3, in reactive astrocytes after the onset of status epilepticus (SE). However, it remains unknown, which molecules act as downstream signals following VEGFR-3 upregulation and are involved in astrogliosis after SE. Therefore, we investigated whether VEGFR-3 upregulation within reactive astrocytes is associated with the activation of mammalian target of rapamycin (mTOR) signaling, which we confirmed by assaying for the phosphorylated form of S6 protein (pS6), and whether VEGFR-3-mediated mTOR activation induces astroglial glutamate transporter-1 (GLT-1) expression in the hippocampus after pilocarpine-induced SE. We found that spatiotemporal expression of pS6 was consistent with VEGFR-3 expression in the hippocampus after SE and that both pS6 and VEGFR-3 were highly expressed in SE-induced reactive astrocytes. Treatment with the mTOR inhibitor rapamycin decreased astroglial VEGFR-3 expression and GLT-1 expression after SE. Treatment with a selective inhibitor for VEGFR-3 attenuated astroglial pS6 expression as well as suppressed GLT-1 expression and astroglial reactivity in the hippocampus after SE. These findings demonstrate that VEGFR-3-mediated mTOR activation could contribute to the regulation of GLT-1 expression in reactive astrocytes during the subacute phase of epilepsy. In conclusion, the present study suggests that VEGFR-3 upregulation in reactive astrocytes may play a role in preventing hyperexcitability induced by continued seizure activity.

Commentary

The vascular endothelial growth factor (VEGF) is a trophic factor that regulates angiogenesis and has neurotrophic and neuroprotective actions in the central and peripheral nervous systems.[1] Signaling through VEGF receptors (VEGFRs 1, 2, or 3) activates downstream signaling cascades such as the phosphatidylinositol-3-kinase (PI3 K)/mechanistic target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2 (ERK) pathways to regulate neurogenesis, neuronal development, and axonal growth as well as survival and proliferation of glial cells.[1] In human and experimental epilepsy, increased expression of VEGFR-3 and enhanced mTOR signaling have been reported in reactive astrocytes.[2] While little is known on the impact that aberrant VEGFR-3 activation has in epilepsy, disruptions in cell-specific roles of mTOR signaling, such as those involved in the regulation of protein synthesis in dendrites, inflammatory/phagocytic properties in microglia, and glutamate transport mechanisms in astrocytes, can promote neuronal instability and hyperexcitability.[3-5] Consequently, mTOR hyperactivation has been linked to epileptogenesis and the generation of unprovoked seizures in both preclinical experimental models and drug-resistant epilepsies in humans.[3-5] Evidence that VEGFR-3 may be an upstream regulator of mTOR suggests that VEGFR-3/mTOR signaling may play a role in astrocytic-mediated epileptogenic processes. Thus, the study by Jeong et al interrogated whether VEGFR-3 signaling mediated mTOR activation as well as the dysregulation of glutamate transporter 1 (GLT-1) and reactive astrocytosis following pilocarpine-induced status epilepticus (SE) in mice.[2]

This study showed that the SE-triggered mTOR pathway activation measured through phosphorylation of the ribosomal S6 protein (P-S6) paralleled the timeline of SE-induced VEGFR-3 increases in the hippocampus. Their comparable spatiotemporal profiles and co-localization in reactive astrocytes suggested a potential role for SE-induced VEGFR-3-mediated mTOR activation in astrogliosis. To investigate this possibility, the authors used rapamycin to inhibit mTOR

activation and SAR131675 (SAR) to block VEGFR-3 signaling between 1 and 3 days after SE. The impact of these treatments was measured through P-S6 and VEGFR-3 levels, and by the expression of the glial fibrillary acidic protein (GFAP) and GLT-1 as markers of astrocytic levels and function, respectively. Rapamycin treatment in the SE group provoked a partial but significant reduction in the levels of P-S6 and in the numbers of VEGFR-3 and GLT-1 positive cells in the hippocampus. Similarly, SAR treatment resulted in a modest but significant decrease in P-S6 levels, while completely attenuating the SE-induced increases in the number of GLT-1 positive cells. This evidence argues in favor of a more substantial role of VEGFR-3 signaling in regulating GLT-1 expression compared to mTOR. However, the role of both mTOR and VEGFR-3 on SE-induced astrogliosis is less clear because GFAP levels were not shown after rapamycin treatment and the effects of SAR in astrogliosis were not fully quantified. Following SAR, the SE-induced increases in the GFAP protein levels were modestly reduced by $\sim$20%, but the actual numbers of GFAP-positive cells were not shown. While the SAR-mediated decreases in both GLT-1 and GFAP suggest that astrogliosis itself was slightly reduced, neither inhibitory treatment completely abolished the aforementioned SE-induced changes. This points to additional or compensatory modulation by perhaps other VEGFR 1-2 or different signaling cascades such as MAPK/ERK.[1] Therefore, the question of whether VEGFR-3 signaling regulates astrogliosis after SE, and to what extent this is mediated by VEGR-3/mTOR activation in astrocytes is still unresolved.

The findings reported by Jeong and colleagues clearly indicate a spatiotemporal association between SE-induced increases in mTOR and VEGFR-3 signaling in the hippocampus that may crosstalk under conditions associated with prolonged seizures. The observation that rapamycin partially suppressed the SE-induced increase in VEGFR-3 suggests that mTOR is also upstream of VEGFR-3. This evidence supports a bidirectional relationship between VEGFR-3 and mTOR, as indicated by the authors. However, this two-way relationship may be further complicated by seizure-induced activation of both VEGF and mTOR signaling in different cell types.[3-7] Thus, a limitation of this study is that it does not provide sufficient evidence to support a direct link between VEGFR-3 and mTOR that is specific to hippocampal astrocytes, as neurons, microglia, or endothelial cells were not examined. Another limitation of this study is that the extent of SAR-mediated suppression on VEGFR-3 expression levels or activity was not demonstrated. Therefore, it is not clear how potent or efficient the impact on VEGF signaling was, or whether it had nonspecific direct effects on VEGFR1/2 or mTOR activation in a cell-type manner. Nevertheless, the findings of this study can set a foundation for future studies to use transgenic approaches to specifically manipulate VEGFR-3 in astrocytes and distinguish from VEGF signaling in neurons or microglia, where VEGF receptors are also abundant.[1]

Overall, the work by Jeong delivers new knowledge supporting that aberrant VEGFR-3 signaling occurs in reactive astrocytes in response to prolonged seizures. However, the extent of the association between VEGFR-3/mTOR signaling in reactive astrocytes, as well as to whether enhanced VEGFR-3 signaling, is pro- or anti-epileptogenic need further investigation. Note that astrogliosis itself is considered a double-edge sword in the construction of epileptic networks because reactive astrocytes may have evolving roles that can promote or prevent epileptogenic processes in a spatiotemporal-dependent manner. In this study, the VEGFR-3-mediated increases in astrocytic GLT1 may reduce neuronal hyperexcitability in the hippocampus by rapidly removing excessive extracellular glutamate,[8] a mechanism that may be compensatory/anti-epileptogenic early after SE. However, the role of VEGF signaling in the context of epilepsy is indeed complex because cell-type specific abnormal VEGF signaling can simultaneously have protective and detrimental consequences. For instance, increased VEGF signaling can promote neuronal and glial survival (anti-epileptogenic) while also disrupting the integrity of the blood brain barrier provoking leakage and unwanted activation of inflammatory cascades (pro-epileptogenic).[1,6,7,9] Thus, to fully understand how exactly VEGF-VEGFR-3 signaling and its crosstalk with mTOR contributes to epilepsy it is necessary to interrogate both pro- versus anti-epileptogenic aspects through cell type-specific and cell signaling–specific manipulations.

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