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Vascular Abnormality in TSC: Leaky Plumbing Is a Problem, but not THE Problem

Cerebral Vascular and Blood Brain-Barrier Abnormalities in a Mouse Model of Epilepsy and Tuberous Sclerosis Complex

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Objective: Tuberous sclerosis complex (TSC) is a genetic disorder, characterized by tumor formation in the brain and other organs, and severe neurological symptoms, such as epilepsy. Abnormal vascular endothelial growth factor (VEGF) expression may promote angiogenesis in kidney and lung tumors in TSC and has been identified in brain specimens from TSC patients, but the role of VEGF and vascular abnormalities in neurological manifestations of TSC is poorly defined. In this study, we investigated abnormalities in brain VEGF expression, cerebral blood vessel anatomy, and blood-brain barrier (BBB) structure and function in a mouse model of TSC. Methods: Tsc1GFAP CKO mice were used to investigate VEGF expression and vascular abnormalities in the brain by Western blotting and immunohistochemical analysis of vascular and BBB markers. In vivo two-photon imaging was used to assess BBB permeability to normally impenetrable fluorescently labeled compounds. The effect of mechanistic target of rapamycin (mTOR) pathway inhibitors, VEGF receptor antagonists (apatinib), or BBB stabilizers (RepSox) was assessed in some of these assays, as well as on seizures by video-electroencephalography. Results: VEGF expression was elevated in cortex of Tsc1GFAP CKO mice, which was reversed by the mTOR inhibitor rapamycin. Tsc1GFAP CKO mice exhibited increased cerebral angiogenesis and vascular complexity in cortex and hippocampus, which were reversed by the VEGF receptor antagonist apatinib. BBB permeability was abnormally increased and BBB-related tight junction proteins occludin and claudin-5 were decreased in Tsc1GFAP CKO mice, also in an apatinib- and RepSox-dependent manner. The BBB stabilizer (RepSox), but not the VEGF receptor antagonist (apatinib), decreased seizures and improved survival in Tsc1GFAP CKO mice. Significance: Increased brain VEGF expression is dependent on mTOR pathway activation and promotes cerebral vascular abnormalities and increased BBB permeability in a mouse model of TSC. BBB modulation may affect epileptogenesis and represent a rational treatment for epilepsy in TSC.

Commentary

Tuberous sclerosis complex (TSC) is a rare life-threatening genetic disease characterized by the growth of noncancerous tumors throughout multiple organs in the body, particularly the lungs, kidneys, and brain. Lesions found in the brain of TSC patients include subependymal nodules and subependymal giant cell astrocytomas, both of which typically form deep within the ependymal lining of the ventricles, and cortical tubers, the namesake of TSC, which form more superficially in the cerebral cortex. These abnormalities in brain anatomy are thought to underlie the severe neurological symptoms of TSC, which include developmental delay, autism, and epilepsy.

In TSC patients with epilepsy, seizures commonly originate from one of the cortical tubers, suggesting a direct link between this anatomical abnormality and one of the disease's most debilitating symptoms. Indeed, surgical resection of well-identified tuber foci is frequently successful at eliminating refractory seizures in TSC patients.[1,2] Thus, cortical tubers themselves represent an obvious therapeutic target for TSC. Unfortunately, the molecular mechanism by which tubers cause seizures (and which might be prophylactically or therapeutically targeted) is unknown. Tuberous sclerosis complex is caused by mutations to the TSC1 or TSC2 gene, either of which disrupts the mechanistic target of rapamycin (mTOR) pathway. However, relevant downstream pathways of mTOR by which cortical tubers promote epileptogenesis have not been identified.

In the highlighted work, Guo et al[3] explore the hypothesis that vascular endothelial growth factor (VEGF) represents a critical pathway in the pathogenesis of TSC. Vascular endothelial growth factor, a subfamily of signaling protein that promotes angiogenesis, is positioned downstream of the mTOR/AP-1 pathway[4] and is implicated in tuber-associated epilepsy

for at least 3 reasons: (1) VEGF is elevated in serum and affected organs in TSC patients,[5,6] (2) in TSC patient tissue, cortical tubers have an elevated density of blood vessels,[7] and (3) blood–brain barrier (BBB) leakage, a consequence of elevated VEGF,[8] has been implicated in tuber-associated epilepsy.[9] Together these findings point to a model wherein mutations to TSC1 or TSC2 genes lead to elevated activity in the mTOR/AP-1/VEGF pathway, which contributes to the formation of ictogenic tubers via increased angiogenesis and increased BBB leakiness.

The authors test this hypothesis in $Tsc1^{GFAP}CKO$ mice, which exhibit inactivation of the TSC1 gene in both neurons and glia, develop seizures after 1 month, and die after 3 to 4 months. While the mice do not develop tubers per se, they replicate many cellular and molecular pathologies of tubers throughout the brain. Here, Tsc1CKO mice were found to have elevated VEGF and hypoxia inducible factor (HIF1α) expression at 4 weeks, both of which were normalized by treatment with rapamycin; thus, mTOR dependent. As in human TSC patients, Tsc1CKO mice exhibited hypervascularization in the brain. This was blocked by a VEGF antagonist, apatinib, suggesting that VEGF is driving the vascular changes. While this study did not directly test whether rapamycin also blocked hypervascularization in Tsc1CKO mice, previous studies have demonstrated that rapamycin can rescue vascular abnormalities in cortical malformations,[7] and it is rational to surmise that blocking the upstream mTOR pathway would have the same effect. Nevertheless, given its clinical relevance, future work may seek to close this gap, as it may be possible to evaluate whether hypervascularity can be treated with mTOR inhibition by comparing clinical measures of vasculature in patients treated with rapamycin analogs (rapalogs) to those not treated.

In addition to the elevated *density* of blood vessels, the authors used in vivo 2-photon imaging of intravascular fluorescent dye to demonstrate that vessels are also *leakier* (measured by presence of extravascular dye). Tight junction proteins, key components of the BBB, were expressed at significantly lower levels in Tsc1CKO mice, further supporting a model of BBB compromise. Blood–brain barrier leakiness (by both measures) was blocked by apatinib and RepSox, a TGFβ antagonist that serves to stabilize the BBB, suggesting that elevated VEGF is also responsible for BBB disruption. Observed anatomical abnormalities in astrocytic endfeet, putatively involved in the BBB disruption, were not measurably altered by apatinib nor RepSox. However such effects may have been more robustly measured with an astrocyte-targeted fluorescent protein, as the dye used in the described experiments (SR101) labels astrocytes via poorly understood (and sometimes nonspecific) active transport and, incidentally can have excitatory side effects.[10] Furthermore, it is possible that enhanced leakiness of the BBB is correlated with leakiness of SR101 from astrocytes (astrocytic somas in the example images look dimmer in Tsc1CKO mice, compared to controls), which would complicate measurement of the very fine end-feet.

Interestingly, while apatinib had no effect on seizures in Tsc1CKO mice, RepSox increased latency to first seizure, decreased seizure frequency, and increased survival compared to vehicle-treated mice. This finding is thought-provoking given that these same dosing paradigms of RepSox and apatanib produced nearly identical effects on vascular leakiness and tight junction protein expression. There are at least 2 possible explanations for this result: (1) RepSox has some BBB-stabilizing or off-target effect that apatinib does not have or (2) apatinib has a proepileptic effect that competes with its putatively anti-epileptic effects of decreasing BBB leakiness and decreasing hypervascularization. One possible way to test this might be to add another experimental group in which both apatinib and RepSox are coadministered. If the effect is the same as RepSox alone, then one might conclude that VEGF acts on an insufficient subset of targets compared to RepSox. If coadministration leads to reduced anti-epilepsy efficacy, then perhaps apatinib has a confounding, proepileptic effect.

Given that rapamycin produces strong anti-epileptic efficacy in this model[11] and apatinib, produces none, one reasonable conclusion from this work might be that, although elevated VEGF leads to hypervascularization and a leaky BBB, these anatomical anomalies do not contribute to epilepsy in this model. If so, identifying the "off-target" effects of RepSox will be critical as they may represent novel targets for anti-epileptic therapies in TSC. One possibility, supported by the finding from this study that HIF1α is elevated in Tsc1CKO mice, is that HIF1α plays an important role in epileptogenesis. It is inhibited by rapamycin and stabilized by TGFβ[12] (primary target of RepSox); and thus, may represent a secondary target of RepSox, which mediates antiepileptic effects upstream and independent of VEGF. Together these interesting, and in some cases unexpected, findings point to a slew of next experiments and novel potential targets for TSC-related epilepsy (with possible implications in post-stroke epilepsy in which vascular changes and HIF1α elevation are also prominent).

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Declaration of Conflicting Interests

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