

PROGRESS TOWARD UNDERSTANDING EPILEPTOGENESIS IN TUBEROUS SCLEROSIS COMPLEX: TWO HITS, NO OUTS, AND THE EKER RAT IS UP TO BAT

Abnormal Cortical Cells and Astrocytomas in the Eker Rat Model of Tuberous Sclerosis Complex

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PURPOSE: In patients with tuberous sclerosis complex (TSC), a wide range of neurologic abnormalities develop, including mental retardation and seizures. Brains from TSC patients are characterized by the presence of cortical tubers, large dysmorphic neurons, and abnormal cytomegalic cells. Although analysis of human TSC brain samples led to the identification of these abnormal cell types, very little is known about how these cells function. In an effort to model TSC-associated CNS abnormalities (and ultimately to analyze the electrophysiologic properties of abnormal cells), we examined Eker rats carrying a *Tsc2* mutation. Anatomic studies, including standard histologic stains and immunocytochemistry, were performed on young Eker rats exposed to a carcinogen in utero or on aged untreated Eker rats (18–24 months old).

METHODS: Pregnant *TSC2*^{+/-} females were injected once a day with hydroquinone, and offspring were killed at postnatal day P14 or P28. Coronal tissue sections throughout the CNS were prepared and stained for cresyl violet. In separate studies, brains of old untreated Eker rats were sectioned for anatomic analysis by using standard immunohistochemical techniques.

RESULTS: Tissue sections stained with cresyl violet did not reveal any gross differences between hydroquinone-treated Eker (*Tsc2*^{E_k/+}) rats and siblings (*Tsc2*^{+/+}). However, two classes of abnormal giant cells were observed in brain sections from untreated aged Eker rats: 1) large dysmorphic pyramid-like cells immunoreactive for NeuN, tuberin, and EAAC-1 in layers IV to VI; and 2) abnormal cytomegalic cells immunoreactive for glial fibrillary acidic protein, vimentin, and nestin in deep cortical layers or along the white matter. In addition, large subependymal astrocytomas were observed in four animals.

CONCLUSIONS: Our data suggest that cortical tuber formation in Eker rats is a rare event and that prenatal exposure to a nongenotoxic carcinogen such as hydroquinone is not sufficient to induce tuber formation. However, with advanced age, an increased likelihood of astrocytoma formation and the emergence of dysmorphic neurons and cytomegalic cells in the Eker rat brain might exist; each of these abnormalities mimics those seen clinically and could contribute to neurologic problems associated with TSC. Further analysis of this rodent model may be warranted.

Morphology of Cerebral Lesions in the Eker Rat Model of Tuberous Sclerosis

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Tuberous sclerosis (TSC) is an autosomal dominant disorder, caused by mutations of either the *TSC1* or *TSC2* gene. Characteristic brain pathologies (including cortical tubers and subependymal hamartomas/giant astrocytomas) are thought to cause epilepsy, as well as other neurologic dysfunction. The Eker rat, which carries a spontaneous germline mutation of the *TSC2* gene (*Tsc2*^{+/+}), provides a unique animal model in which to study the relation between

TSC cortical pathologies and epilepsy. In the present study, we analyzed the seizure propensity and histopathologic features of a modified Eker rat preparation, in which early postnatal irradiation was used as a “second hit” stimulus in an attempt to exacerbate cortical malformations and increase seizure propensity. Irradiated Eker rats had a tendency toward lower seizure thresholds (latencies to flurothyl-induced seizures) than seen in nonirradiated Eker

rats (significant difference) or irradiated wild-type rats (nonsignificant difference). The majority of irradiated Eker rats exhibited dysplastic cytomegalic neurons and giant astrocyte-like cells, similar to cytopathologies observed in TSC lesions of patients. The most prominent features in these brains were hamartoma-like lesions involving large eosinophilic cells, similar to giant tuber cells in human

TSC. In some cells from these hamartomas, immunocytochemistry revealed features of both neuronal and glial phenotypes, suggesting an undifferentiated or immature cell population. Both normal-appearing and dysmorphic neurons, as well as cells in the hamartomas, exhibited immunopositivity for tuberin, the protein product of the TSC2 gene.

COMMENTARY

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder caused by mutation of one of two genes: *Tsc1*, which produces the protein hamartin, and *Tsc2*, which codes for the protein tuberin. Hamartin and tuberin are thought to be regulators of cell signaling and growth, and dysfunction of these proteins underlies the abnormal growth regulation in many organ systems in TSC (1,2). In TSC, tumors arise in the heart, kidney, lungs, and other structures, but the brain is often the most seriously affected organ. Individuals with TSC often have intractable epilepsy, mental retardation, and autism. Brain pathology in TSC includes a variety of abnormalities, including cortical tubers, which are disorganized, hamartomatous cellular regions, and subependymal nodules. Subependymal nodules can transform into giant cell astrocytomas, which often lead to acute neurologic dysfunction, such as obstructive hydrocephalus. These neuropathologic entities reflect aberrant neuronal migration and differentiation; some dysmorphic cells in TSC contain mixed neuronal and glial markers.

Epilepsy is an almost universal manifestation of TSC, with more than 90% of affected individuals having seizures ranging from infantile spasms to complex partial seizures (3). Seizures often arise from neuronal tissue directly adjacent to cortical tubers, and tuber removal is often curative of the seizures (4). Our understanding of epileptogenesis in TSC is limited by the lack of an animal model that replicates the pathology of the human disorder (5). The ideal animal model would reproduce the genetic defect, structural brain abnormalities, and clinical features of human TSC. Some animal models have been created by targeted deletions of the *Tsc1* or *Tsc2* genes. Homozygous TSC gene mutations are typically lethal during embryogenesis, whereas brains of heterozygous animals rarely reveal the typical neuropathology of human TSC. Therefore, in heterozygous mutations, “second hits” are often used to reproduce more faithfully the pathologic conditions of TSC by producing a “loss of heterozygosity.” In animals with a conditional knockout of *Tsc1* or *Tsc2*, abnormal cytopathology develops, including hippocampal disorganization, astrocyte proliferation, and cortical

dyslaminar; however, typical cortical tubers have not been reported (6). *TSC1* knockouts do express spontaneous seizures by 2 months of age, which possibly is due to altered glial glutamate transport (7).

The Eker rat is a spontaneous germline mutation model of TSC, in which one TSC2 allele is inactivated. This mutation results in abnormal *Tsc2* gene product, tuberin, which is a guanosine triphosphatase-activating protein. Renal tumors develop in Eker rats (8), but the abnormal neuronal types of human TSC have been difficult to confirm. Over several decades of research using Eker rats, minimal neurologic deficits have been reported. Only a single Eker rat has ever been shown to have a typical cortical tuber (9). One theory of TSC pathogenesis invokes the second-hit hypothesis, whereby an initial germline mutation is compounded by a second, subsequent somatic mutation. Both of the studies reviewed here use second hits in the Eker rat in attempts to create a more realistic TSC model.

In the article by Takahashi and colleagues, pregnant rats with a *TSC2*^{+/-} genotype were exposed to hydroquinone, a carcinogen known to produce renal carcinomas in Eker carriers, which was the designated second hit. *Tsc2*^{Ek/+} offspring, killed on postnatal days 14 or 28, had no histologic abnormalities compared with *Tsc2*^{+/+} siblings. Therefore, hydroquinone does not replicate the neuropathology of human TSC. The authors next examined aged Eker rats, in which advanced age was considered the second hit. In these aged rats, several histologic features resembled the human TSC pathology, including large dysmorphic pyramidal neurons in deep cortical layers (immunoreactive for neuronal markers), abnormal cytomegalic cells (immunoreactive for glial markers), and glial tumors in a proportion of aged rats. The presence of these abnormal cells in nontuber cortex suggests that they might form an abnormally excitable network that could underlie seizure generation. None of these rats had observed seizures, but seizures were not assayed systematically or electrographically. The authors hope to examine the tissue from these Eker rats in vitro to explore its epileptogenic properties, but they point out that the paucity of abnormal neurons would make electrophysiologic identification and recording quite challenging. Perhaps a large number

of such dysmorphic neural elements must be present to generate seizures. One caveat of this model's validity for TSC is that in humans, TSC manifestations are often present in very young patients, not only at older ages. Nevertheless, this article demonstrates that Eker rats have at least some neuropathologic similarities to the human, making them attractive to study epilepsy in greater depth.

Wenzel and colleagues took a different approach, by using early postnatal irradiation (days 0 to 3) as the second hit. In *Tsc2*^{+/-} Eker rats, but not in wild-type rats, irradiation resulted in a smaller dentate granule cell layer. Eker rats displayed dysmorphic large neurons, giant astrocyte-like cells, and subependymal hamartomas, none of which were present in non-irradiated Eker rats or irradiated wild-type rats. However, no cortical tubers or subependymal giant cell astrocytomas were found. In the hamartoma-like lesions, many cells had both neuronal and glial features and expressed tuberin. Perhaps most intriguing, Eker rats that received early radiation exposure had shorter latencies to flurothyl-induced seizures than did both nonirradiated Eker rats and irradiated wild-type rats (although the latter did not quite reach statistical significance). None of the rats had spontaneous seizures. These results suggest that the irradiated Eker rat possesses many similar neuropathologic features of TSC and a tendency toward hyperexcitable network activity, making it an attractive potential model to study epileptogenesis.

These two reports suggest that second-hit models of the Eker rat may yet model epilepsy in TSC. The lack of typical tuber formation limits their generalizability to TSC, but Eker rats do display neuropathologic characteristics that are reminiscent of TSC. Whether these or other second hits will predispose the animals to a more robust seizure propensity remains to be clarified, but an urgent need exists to understand better why persons with TSC are so seizure prone and how to ameliorate

their epilepsy. In this regard, we are still in the early innings of the game.

by Carl E. Stafstrom, MD, PhD

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